Universidad CEU San Pablo

CEINDO – CEU Escuela Internacional de Doctorado

PROGRAMA en CIENCIA Y TECNOLOGÍA DE LA SALUD

Efecto de una dieta rica en grasa saturada durante la adolescencia sobre la transmisión glutamatérgica hipocampal. Relación con la leptina y la disfunción endocrina del tejido adiposo

TESIS DOCTORAL

Presentada por:

Jesús Fernández Felipe

Dirigida por:

Dr. Mariano Ruiz Gayo

Dra. Beatriz Merino Palacios

MADRID, 2022

La presente tesis, titulada '**Efecto de una dieta rica en grasa saturada durante la adolescencia sobre la transmisión glutamatérgica hipocampal. Relación con la leptina y la disfunción endocrina del tejido adiposo**' se presenta como un compendio de trabajos publicados y cumple las condiciones exigidas por la normativa establecida para este tipo de tesis, aprobada por el comité de dirección de la CEINDO el 30 de noviembre de 2017.

Las publicaciones y la bibliometría de las revistas en las que se han publicados son las siguientes:

1. **Título:** *Saturated and unsaturated fat diets impair hippocampal glutamatergic transmission in adolescent mice*.
   Autores: Jesús Fernández Felipe, Beatriz Merino, Ana Belén Sanz Martos, Adrián Plaza, Ana Contreras, Víctor Naranjo, Julie Chowen, Victoria Cano, Mariano Ruiz Gayo y Nuria del Olmo.
   Publicado en: *Psychoneuroendocrinology* (ISSN: 0306-4530)
   Factor de impacto: 4.693
   Cuartil: Q2 (neurociencia)
   Fecha de publicación: 2021
   DOI: 10.1016/j.psyneuen.2021.105429
2. **Título:** *Effects of saturated versus unsaturated fatty acids on metabolism, gliosis, and hypothalamic leptin sensitivity in male mice*.
   Autores: Jesús Fernández Felipe, María Valencia Avezuela, Beatriz Merino, Beatriz Somoza, Victoria Cano, Ana Belén Sanz Martos, Laura Frago, María Soledad Fernández Alfonso, Mariano Ruiz Gayo y Julie Chowen
   Publicado en: *Nutritional neuroscience* (1028-415X)
   Factor de impacto: 4.062

Cuartil: Q2 (neurociencia)

Fecha de publicación: 2022

DOI: 10.1080/1028415X.2022.2029294

3. **Título:** *Effect of Lauric vs. Oleic Acid-Enriched Diets on Leptin Autoparacrine Signalling in Male Mice*.

   Autores: Jesús Fernández Felipe, Adrián Plaza, Gema Domínguez, Javier Pérez Castells, Victoria Cano, Francesco Cioni, Nuria del Olmo, Mariano Ruiz Gayo y Beatriz Merino.

   Publicado en: *Biomedicines*

   Factor de impacto: 4.757

   Cuartil: Q2 (bioquímica y biología molecular)

   Fecha de publicación: 2022

   DOI: 10.3390/biomedicines10081864

D. **MARIANO RUIZ GAYO**, catedrático del Departamento de Ciencias Farmacéuticas y de la Salud de la Facultad de Farmacia de la Universidad CEU San Pablo y Dña. **BEATRIZ MERINO PALACIOS**, profesora titular del Departamento de Ciencias Farmacéuticas y de la Salud de la Facultad de Farmacia de la Universidad CEU-San Pablo

HACEN CONSTAR:

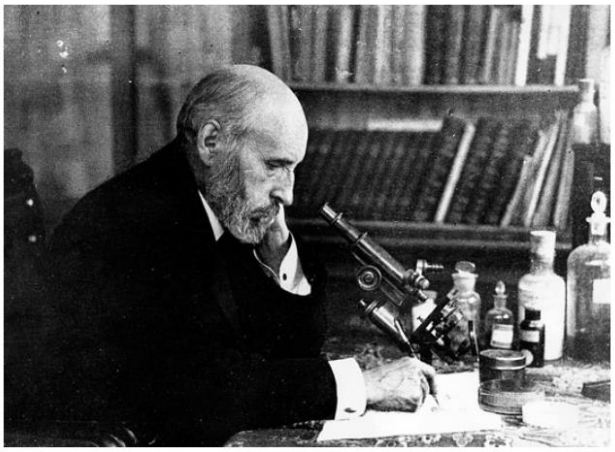
Que D. **JESÚS FERNÁNDEZ FELIPE** ha realizado bajo su dirección el trabajo titulado “Efecto de una dieta rica en grasa saturada durante la adolescencia sobre la transmisión glutamatérgica hipocampal. Relación con la leptina y la disfunción endocrina del tejido adiposo” con objeto de obtener el Grado de Doctor, y que dicho trabajo reúne todos los requisitos necesarios para su juicio y calificación.

Madrid, a 23 de noviembre de 2022

Fdo.: Mariano Ruiz Gayo

Fdo.: Beatriz Merino Palacios

Este trabajo ha sido financiado por el Ministerio de Ciencia e Innovación (BFU2016-78556R y PID2020-117422RB-C21/AEI/10.13039/501100011033), el Fondo Europeo de Desarrollo Regional y la Fundación Universitaria San Pablo-CEU. Jesús Fernández Felipe ha sido beneficiario de una ayuda para la formación de personal de investigación (FPI) de la Fundación Universitaria San Pablo-CEU desde octubre de 2018 hasta octubre de 2022. También ha disfrutado de la ayuda a la movilidad CEU-Banco Santander, con la que ha podido realizar una movilidad investigadora en la Universidad de Copenhague desde abril hasta junio de 2022.



"Todo ser humano, si se lo propone, puede ser escultor de su propio cerebro"

Santiago Ramón y Cajal

ÍNDICE GENERAL

RESUMEN

La **leptina** es una hormona de acción pleiotrópica que se sintetiza en el tejido adiposo. Existe un eje tejido adiposo-cerebro puesto que los receptores de leptina se expresan por todo el sistema nervioso central y participan en el control de funciones cognitivas (**hipocampo**) y en la regulación neuroendocrina de la ingesta (**hipotálamo**). El consumo de dietas ricas en grasas produce un desequilibrio en el **tejido adiposo** que conduce a un aumento de la producción de leptina y a hiperleptinemia, capaz de desensibilizar el receptor de leptina (RLep) y de producir deterioro cognitivo y desequilibrio entre ingesta y gasto energético. Sin embargo, no se conoce la contribución de cada componente de la dieta (azúcar, grasas saturadas, grasas insaturadas) a estas alteraciones. El **objetivo** de este trabajo ha sido identificar la influencia específica de las grasas saturadas y monoinsaturadas en la funcionalidad del RLep en el tejido adiposo, el hipotálamo y el hipocampo. Para ello, se han utilizado ratones macho C57BL/6J alimentados con una dieta enriquecida en grasas saturadas (SOLF) o monoinsaturadas (UOLF) durante 8 semanas. Nuestros **resultados** muestran que la ingesta de grasas saturadas y monoinsaturadas deteriora la plasticidad sináptica, aunque de forma diferente. Mientras SOLF reduce la LTP y bloquea la recuperación de la LTD, UOLF impide el mantenimiento de la LTP y anula la LTD. Además, se observa una disminución concomitante de la expresión de las subunidades de los receptores de glutamato, AMPA-1, NMDA-2A y NMDA-2B, y del receptor PPARγ, sobre todo en los animales que consumieron SOLF. En estos animales también se produce un deterioro de la memoria espacial dependiente de hipocampo. Por otra parte, mientras que ambas dietas aumentan la concentración de glutamato y aspartato y disminuyen la de glutamina, sólo UOLF reduce los niveles de D-serina en el hipocampo. Respecto a la señalización del RLep en el hipocampo, la dieta SOLF altera la vía de la STAT3, mientras que la dieta UOLF desensibiliza las vías de la Akt y la AMPK. En cambio, en el hipotálamo, estas dos vías se bloquean por la dieta SOLF. En el tejido adiposo, ambas dietas producen hipertrofia, aunque la expresión de leptina y la leptina plasmática solo aumentan con la dieta UOLF. Sin embargo, solamente la dieta SOLF desacopla el RLep de las vías STAT3, Akt y AMPK. En **conclusión**, nuestro estudio demuestra que la composición de la dieta

determina el grado de disfunción hipocampal y de resistencia a leptina. Así, el consumo crónico de ambas dietas interfiere en el funcionamiento del hipocampo, pero el consumo de grasas saturadas es capaz de alterar la señalización del receptor de leptina en el tejido adiposo, el hipocampo y el hipotálamo. Nuestro estudio sugiere que el ácido láurico, un componente mayoritario de SOLF, podría ser responsable de parte de los efectos inducidos por esta dieta.

ÍNDICE DE FIGURAS

ÍNDICE DE TABLAS

ABREVIATURAS

ACTH	**Adrenocorticotropina**
AgRP	***Agouti-related peptide***
AL	**Ácido Láurico**
AMPA	**Ácido α-amino-3-hidroxi-5-metil-4-isoxazolepropiónico**
AMPK	**Proteína quinasa activada por AMP**
AO	**Ácido oleico**
AP	**Ácido palmítico**
CA	***Cornu ammonis***
DM-II	**Diabetes *mellitus* tipo 2**
D-Ser	**D-Serina**
EA	**Enfermedad de Alzheimer**
FA	***Fatty acid***
GABA	**Ácido gamma-aminobutírico**
GD	**Giro dentado**
Gly	**Glicina**
HFD	**Dietas ricas en grasa**
IL	**Interleuquina**
JAK	**Quinasa Janus**
LCFA	**Ácido graso de cadena larga**
L-Gln	**L-Glutamina**
L-Glu	**L-Glutamato**
L-Ser	**L-Serina**
LTD	***Long-term depression***
LTP	***Long-term potentiation***
MCFA	**Ácido graso de cadena media**
MCH	**Hormona concentrante de melanina**
MCP-1	**Proteína quimioatrayente de monocitos 1**
MCT	**Triglicérido de cadena media**
MUFA	**Ácidos grasos monoinsaturados**
NEFA	**Ácidos grasos no esterificados**
NMDA	**Ácido N-metil-D-aspártico**

NPY	**Neuropéptido Y**
PI3K	**Quinasa de fosfoinosítidos 3**
POMC	**Proopiomelanocortina**
PPAR	**Receptor de activadores de la proliferación de peroxisomas**
PUFA	**Ácidos grasos poliinsaturados**
RLep	**Receptor de leptina**
SCFA	**Ácido graso de cadena corta**
SNC	**Sistema nervioso central**
SOCS3	**Supresor de la señalización de citoquinas 3**
SOLF	***Saturated oil-enriched food***
SRR	**Serina racemasa**
STAT3	***Signal transducer and activator of transcription 3***
TA	**Tejido adiposo**
TAB	**Tejido adiposo blanco**
TAM	**Tejido adiposo marrón**
TNF-α	**Factor de necrosis tumoral alfa**
UOLF	***Unsaturated oil-enriched food***
αMSH	**Hormona estimulante de melanocitos alfa**

INTRODUCCIÓN

PREÁMBULO

El consumo de dietas ricas en grasa (*High Fat Diets*, HFDs), junto con el sedentarismo, son considerados los factores más importantes implicados en el desarrollo de sobrepeso y obesidad. Desde 1975, el número de personas obesas en el mundo se ha triplicado. De hecho, se estima que hoy día hay más de 1900 millones de personas mayores de 18 años con estas patologías. Si nos centramos en la obesidad infantil, los datos son incluso más preocupantes, ya que desde 1975 el porcentaje de sobrepeso en niños ha aumentado del 4 al 18% (**Hamilton y cols., 2007**; **OMS, 2018**).

El sobrepeso y la obesidad están dentro de las alteraciones nutricionales más comunes durante la niñez y la adolescencia, siendo las principales causas los cambios en los hábitos alimenticios y la composición de la dieta. En las últimas décadas se ha producido un incremento en el consumo de las llamadas dietas *western*, con gran contenido en azúcares y grasas saturadas, debido principalmente a sus propiedades organolépticas y de palatabilidad. Esto se ha acompañado de una disminución en la actividad física, promoviéndose el desarrollo de sobrepeso y obesidad en este grupo poblacional (**Francis and Stevenson, 2013; OMS, 2021**).

Diversos estudios han demostrado que la aparición de obesidad en edades tempranas aumenta el riesgo de ésta en la edad adulta, lo que se acompaña de una mayor incidencia de enfermedades metabólicas y cardiovasculares. Así, la obesidad infantil se ha relacionado con enfermedades gastrointestinales, musculoesqueléticas, apnea del sueño y otras patologías como hipertensión, dislipemias y diabetes mellitus tipo II (DM-II) (**Han y cols., 2010; Vázquez y cols., 2007; OMS, 2021**).

Por otro lado, los pacientes con obesidad sufren un mayor deterioro del sistema nervioso central (SNC) y de las funciones cognitivas. En este sentido, se ha visto que la ingesta de HFDs aumenta la probabilidad de desarrollar enfermedades neurodegenerativas como enfermedad de Alzheimer (EA) o demencia (**Nilsson y Nilsson, 2009**). De hecho, algunos estudios sugieren que las alteraciones cognitivas son, en parte, consecuencia de las enfermedades

metabólicas asociadas a la obesidad, como la DM-II o la hipertensión (**van den Berg y cols., 2009**), lo que se conoce como diabetes tipo 3 (**De Felice y Ferreira, 2014; de la Monte y Wands, 2008; Kandimalla y cols., 2017**). En este sentido, estudios anteriores de nuestro laboratorio han demostrado en roedores que el consumo de HFDs durante la adolescencia tiene un efecto perjudicial sobre el aprendizaje y la memoria dependientes de **hipocampo**, que se acompaña de **hiperleptinemia** y resistencia a la leptina en dicha área cerebral (**Caprio y cols., 1996; Myers y cols., 2010; Valladolid-Acebes y cols., 2013, 2012, 2011**). Estas dietas presentan, además, una elevada cantidad de azúcares, lo que dificulta la asociación directa entre el consumo de grasas y los posibles efectos nocivos de las HFDs. Surge, por tanto, la pregunta sobre cuál de los componentes de estas dietas es el verdaderamente responsable de dichas alteraciones.

La leptina, por otra parte, es una adipocitoquina secretada por el tejido adiposo (TA), esencial en el control de la homeostasis energética, que parece tener un papel decisivo en el correcto funcionamiento neuronal. En los últimos años, se ha puesto de manifiesto la participación de esta hormona en áreas como el hipocampo, implicada en la memoria y el aprendizaje (**Harvey, 2007**), así como en el **hipotálamo**, esencial en la regulación de la ingesta (**Vaissey cols., 1996**), señalando a la leptina como el eje central del **eje adiposo-SNC** (**Shimizu y Mori, 2005**). De esta manera, algunos autores proponen que la resistencia a la leptina inducida por el consumo de HFDs podría explicar los déficits cognitivos inducidos por la ingesta de este tipo de alimentos (**Morrison, 2009**). Por lo tanto, la resistencia a leptina podría ser un factor clave en las alteraciones de la memoria observadas tras el consumo de dietas hipercalóricas (**Dinel y cols., 2011; L. J. Shanley y cols., 2001**).

Teniendo en cuenta estos antecedentes, el objetivo principal de esta Tesis Doctoral ha sido el efecto de un consumo elevado de grasas en el SNC, así como los desajustes en el eje adiposo-SNC asociados a la hiperleptinemia secundaria al consumo de dichas grasas. Para llevar a cabo este estudio, nuestro laboratorio ha desarrollado dos tipos de dieta con alto contenido en grasas, pero no en azúcares: una dieta rica en grasas saturadas, con **alto contenido en ácido**

palmítico y ácido láurico, denominada **SOLF** (*Saturated OiL-enriched Food*) y otra **rica en ácido oleico**, denominada **UOLF** (*Unsaturated OiL-enriched Food*).

1. DIETAS OCCIDENTALES

Las enfermedades no transmisibles, como las enfermedades cardiovasculares, el cáncer, las enfermedades respiratorias crónicas, la diabetes, la obesidad y el deterioro cognitivo, se encuentran entre las principales causas de muerte y discapacidad en todo el mundo, afectando tanto a países desarrollados como en vías de desarrollo (**OMS, 2021b**). Aunque los factores genéticos y ambientales contribuyen en el desarrollo de estas patologías, los factores modificables del estilo de vida como la dieta desempeñan un papel importante (**Budreviciute y cols., 2020; Mikkelsen y cols., 2019**). Se ha demostrado que la dieta aumenta el riesgo de hipertensión, hipercolesterolemia, sobrepeso/obesidad, diabetes y cáncer, entre otras enfermedades (**Aune y cols., 2009; Eaton y cols., 1988; Imamura y cols., 2015**). De hecho, el aumento exponencial de pacientes con estas enfermedades se ha relacionado con una mayor ingesta de las llamadas dietas occidentales caracterizadas por su alto contenido en proteínas de origen animal, grasas saturadas, cereales refinados, sal y azúcares (**Cordain y cols., 2005; Kopp, 2019**).

La ingesta excesiva de grasas favorece la hiperplasia e hipertrofia del TA, lo que desencadena cambios funcionales y/o estructurales en el tejido, que aumenta la secreción de leptina, de adipoquinas pro-inflamatorias como el TNF-α o la IL-6, mientras que disminuye la producción de adipoquinas antiinflamatorias como la adiponectina (**Forny-Germano y cols., 2018**). La desregulación hormonal del tejido adiposo, junto con la capacidad del TNF-α y la IL-6 de atravesar la barrera hematoencefálica, aumentan el riesgo de neuroinflamación asociada al deterioro cognitivo. Este hecho pone de manifiesto la importante comunicación entre el TA y el SNC (**Aguilar-Valles y cols., 2015; Ferreira y cols., 2014**). Del mismo modo, otros estudios demuestran que el consumo prolongado de HFDs conduce a un estado inflamatorio característico de la obesidad, provocando la disfunción de áreas cerebrales implicadas en la regulación de la ingesta, el estado de ánimo, el aprendizaje y la memoria, como el hipotálamo y el hipocampo (**Castanon y cols., 2015, 2014; Dalvi y cols., 2017**).

1.1 Tipos de ácidos grasos presentes en las dietas occidentales

Los ácidos grasos (*Fatty acids,* FA*)* son nutrientes esenciales para la mayoría de las células, ya que se utilizan como combustible y son un sustrato esencial para el metabolismo de los fosfolípidos. Se clasifican según la longitud de la cadena de carbono en ácidos grasos de cadena corta (*Short Chain Fatty Acid*, SCFA) (<6 C), ácidos grasos de cadena media (*Medium Chain Fatty Acid*, MCFA) (entre 8 C y 12 C) y ácidos grasos de cadena larga (*Long Chain Fatty Acid*, LCFA) (entre 14 C y 18 C). Además, los ácidos grasos también se clasifican en función del número de dobles enlaces, por lo que tenemos ácidos grasos saturados, que carecen de dobles enlaces, monoinsaturados (*MonoUnsaturated Fatty Acid*, MUFA) con un doble enlace y poliinsaturados (*PolyUnsaturated Fatty Acid*, PUFA) con más de un doble enlace (**Tvrzicka y cols., 2011**) (Tabla 1).

Tabla 1. Clasificación de los ácidos grasos según su longitud y el número de insaturaciones

Longitud de la cadena	Clasificación por longitud	Clasificación por insaturación	Nombro propio
C4:0	SCFA	Saturado	Ácido butírico
C6:0	SCFA	Saturado	Ácido caproico
C10:0	MCFA	Saturado	Ácido cáprico
C12:0	MCFA	Saturado	Ácido láurico
C16:0	LCFA	Saturado	Ácido palmítico
C18:0	LCFA	Saturado	Ácido esteárico
C18:1Δ^{9}	LCFA	MUFA	Ácido oleico
C18:2$\Delta^{9,12}$	LCFA	PUFA	Ácido linoleico

Un estudio realizado en 1996 por Greenwood y Winocur comparó el efecto de varias dietas con diferente composición en ácidos grasos saturados, MUFA y PUFA sobre la función cognitiva, y mostró que la contribución al deterioro cognitivo era mayor en las dietas con un mayor porcentaje de ácidos grasos saturados (**Greenwood y Winocur, 1996**). Sin embargo, es importante señalar que la mayoría de los estudios que evalúan el efecto perjudicial de las HFDs sobre la cognición se han llevado a cabo con dietas grasas comerciales, con un alto contenido en sacarosa y grasa, especialmente manteca de cerdo (45-60%

de la dieta), y con una mayor proporción de ácidos grasos saturados que insaturados (**Boitard y cols., 2012, Del Rio y cols., 2016, Valladolid-Acebes y cols., 2013, Kaczmarczyk y cols., 2013**).

A pesar de que estas dietas son similares a las consumidas en las sociedades occidentales, es importante conocer si los déficits cognitivos y metabólicos observados en estos estudios son consecuencia de la sacarosa, de los ácidos grasos saturados/ insaturados o de una sinergia entre todos sus componentes. Por este motivo, en los últimos años han aumentado el número de estudios centrados en evaluar el efecto de los ácidos grasos, saturados e insaturados, y de los azúcares sobre los procesos cognitivos y la homeostasis energética.

En cuanto al consumo particular de ácidos grasos, está descrito que existen diferencias cuanti- y cualitativas dependiendo del ácido graso ingerido. Así, se ha visto que la ingesta de dietas enriquecidas en grasas saturadas altera la ingesta y el comportamiento (**Kaplan y Greenwood, 1998**), así como la morfología hipocampal y la memoria (**Matura y cols., 2021**), llegando a ser un factor de riesgo para desarrollar demencia (**Morris y Tangney, 2014**). Por otra parte, el consumo de dietas enriquecidas en grasas insaturadas parece ejercer un efecto beneficioso tanto a nivel metabólico como cerebral, mejorando los niveles de glucosa e insulina y revirtiendo los efectos perjudiciales de las dietas ricas en grasas saturadas o en azúcares (**Lim y cols., 2010; Tay y cols., 2018**). Sin embargo, en las últimas décadas se ha demostrado que no todos los ácidos grasos insaturados tienen efectos beneficiosos. Así, los PUFA ω-3 parecen activar vías de señalización antiinflamatorias, mejorando las alteraciones metabólicas y cognitivas observadas en modelos animales de Alzheimer (**Simopoulos, 2016**), mientras que los PUFA ω-6 y algunos MUFA se han descrito como perjudiciales, puesto que promueven la inflamación en tejidos como el TA o el hipotálamo, llegando a interferir en la respuesta de hormonas como la insulina (**González-Becerra y cols., 2019; Ravaut y cols., 2020**).

Desde el punto de vista nutricional, los efectos nocivos observados sobre estos tejidos por el consumo de dietas ricas en grasa podrían deberse a la combinación de diferentes ácidos grasos, quedando en duda el papel de cada uno de ellos. Por este motivo, en nuestro grupo de investigación hemos diseñado

dos dietas bajas en carbohidratos y enriquecidas en grasas saturadas (SOLF, con alto contenido en **ácidos palmítico** y **láurico**) e insaturadas (UOLF, rica en **ácido oleico** (Tabla 2) (**Plaza y cols., 2019**), con el fin de determinar el efecto de las grasas, sin interferencia de los azúcares.

Tabla 2. Composición de las dietas

Composición	SD	SOLF	UOLF
Densidad energética (kcal/g)	3.09	5.27	5.30
Proteínas totales (%)	21.01	13.12	12.96
Carbohidratos totales (%)	42.89	27.07	26.90
Lípidos totales (%)	6.01	40.82	41.17
Grasa saturada (%)		78	22
Grasa insaturada (%)		10	90
Ácido palmítico (%)	0.68	6.07	2.97
Ácido oleico (%)	1.16	10.04	31.29
Ácido láurico (%)	-	19.85	-
Otros FAs (%)	4.17	5.21	6.13

1.2 Ácidos grasos presentes en SOLF y UOLF

El ácido oleico (C18:1; ω-9) es un MUFA de 18 carbonos (LCFA) con un doble enlace entre los carbonos 9 y 10. El consumo de MUFA como parte de la ingesta de grasa dietética representa al menos un tercio de la ingesta total de ácidos grasos en el área mediterránea, donde las principales grasas culinarias utilizadas son los aceites de oliva y girasol, cuyo principal ácido graso es precisamente el ácido oleico. Diversos estudios han demostrado los beneficios de este tipo de grasa sobre la salud, debido en gran parte al efecto del ácido oleico en la reducción de los niveles de colesterol o el riesgo de aterosclerosis, junto a su efecto inmunomodulador y antiinflamatorio. Estos efectos sugieren que el ácido oleico es un componente dietético esencial en el tratamiento y prevención de enfermedades cardiovasculares, autoinmunes, trastornos metabólicos y cáncer (**Sales-Campos y cols., 2013**). Por otra parte, también se ha demostrado que el ácido oleico ejerce efectos beneficiosos sobre el SNC. En

este sentido, se ha descrito que el ácido oleico tiene un papel neuroprotector, no sólo en modelos sanos **(Bento-Abreu y cols., 2007; Tabernero y cols., 2001**) sino también en modelos murinos de EA (**Amtul y cols., 2011**). Estos resultados podrían estar mediados por la unión del ácido oleico al PPARα y en este sentido, esa puede ser la razón por la que los MUFA modulan la expresión de los péptidos orexigénicos en el hipotálamo (**Jagannathan y cols., 2020**). Sin embargo, los estudios han demostrado que el verdadero efecto beneficioso se produce cuando existe una proporción equilibrada de PUFA/MUFA. De esta manera, un aumento de los niveles de ácidos grasos tipo ω-6 y 9 con respecto a los tipo ω-3, parece ser perjudicial, sobre todo para el SNC (**Wysoczański y cols., 2016**).

Por otra parte, el ácido palmítico (C16:0), un ácido graso saturado y de cadena larga, parece promover procesos neuroinflamatorios. Se ha demostrado que estimula la liberación de citoquinas proinflamatorias como el TNF-α y la IL-6 en los astrocitos (**Dalvi y cols., 2017; Gupta y cols., 2012**), además de tener un efecto lipotóxico debido al aumento de los niveles de ROS, lo que reduce la viabilidad de las células progenitoras neuronales y reduce la neurogénesis del hipocampo (**Gupta y cols., 2012; Park y cols., 2011**). Por otro lado, se ha descrito que el ácido palmítico está involucrado en las alteraciones que ocurren en la EA, ya que induce la síntesis de ceramidas a nivel astroglial **(Patil y cols., 2008, 2007, 2006**). Además, se ha descrito que el ácido palmítico es capaz de modular la sensibilidad a la leptina en el SNC, lo que podría explicar los efectos centrales de la HFDs (**Cheng y cols., 2015; Keung y cols., 2011**).

Por último, la dieta SOLF también tiene un contenido elevado de ácido láurico (C12:0), que es un ácido graso saturado de cadena media (MCFA) que se encuentra principalmente en los aceites de coco y palmiste (**Patil y cols., 2008, 2006**). Al igual que ocurre con el ácido palmítico, el ácido láurico también aumenta la liberación de citoquinas proinflamatorias como el TNF-α y la IL-6 en los astrocitos (**Gupta y cols., 2012**) y promueve la síntesis de cuerpos cetónicos (**Nonaka y cols., 2016**). Sin embargo, las dietas ricas en ácido láurico se están utilizando en las dietas de adelgazamiento y también para el tratamiento de cuadros convulsivos y del deterioro cognitivo en la EA (**Liu y Wang, 2013; Ota y cols., 2019**). En primer lugar, está descrito que estas dietas disminuyen el peso corporal ya que el ácido láurico no sólo no se acumula en el TA sino que parece

promover lipólisis, mejorando así su sensibilidad a insulina en modelos de obesidad (**Xia y cols., 2021**). Además, se ha observado que el consumo de dietas enriquecidas en ácidos grasos de cadena media, y en particular de ácido láurico, aumentan los niveles plasmáticos de cuerpos cetónicos, los cuales es conocido su efecto beneficioso sobre el SNC (**Jensen y cols., 2020; McCarty y DiNicolantonio, 2016**). Sin embargo, no se conocen los mecanismos involucrados en la mejoría de los animales con deterioro cognitivo y menos aún los efectos sobre diferentes áreas cerebrales particulares como el hipocampo o el hipotálamo.

2. HIPOCAMPO

2.1 Estructura y conexiones

El hipocampo es una estructura cerebral que forma parte del sistema límbico, cuyas dos mitades se sitúan en la región medial del lóbulo temporal de ambos hemisferios. Las conexiones neuronales intrahipocampales siguen una organización laminar definida, en la que cada estrato de neuronas piramidales establece vías conectivas unidireccionales. Dentro del hipocampo, el flujo de información se propaga a través de las capas superficiales de neuronas piramidales compactas desde la circunvolución dentada hasta la corteza entorrinal, pasando por las regiones CA1 y CA3. Cada una de estas capas tiene un sistema de comunicación neuronal intrínseco muy complejo con un gran número de conexiones longitudinales (**Amaral y Witter, 1989; Leranth y Hajszan, 2007**).

El hipocampo recibe conexiones aferentes de tres vías principales, i) la vía perforante, que hace sinapsis con las células granulares del giro dentado, ii) la vía de las fibras musgosas, que establece conexiones entre el giro dentado (GD) y el área CA3 y iii) las fibras comisurales colaterales de Schaffer, que se proyectan a la región CA1 (Figura 1) (**Kobayashi, 2010; Szirmai y cols., 2012; Witter, 2007**).

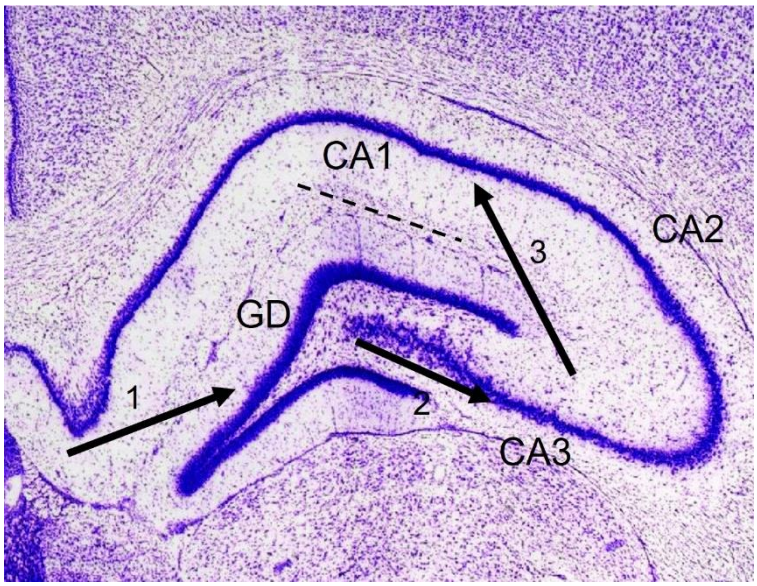


Figura 1. Áreas del hipocampo y vías de entrada de información (*adaptado de* ***Paxinos y Watson 2007).*** *La información accede al hipocampo a través de la vía perforante (1) hacia el GD. A continuación, las neuronas granulares del GD hacen sinapsis con las neuronas piramidales de CA3 mediante las fibras musgosas (2). Finalmente, las neuronas piramidales de CA3 conectan con las piramidales de CA1 haciendo uso de las fibras colaterales de Schaffer (3). Dentro de CA1, la línea discontinua separa los cuerpos neuronales (por encima de ella) de los axones (por debajo de ella).*

Además, el hipocampo presenta conexiones eferentes con otras áreas cerebrales, a través de proyecciones indirectas y circuitos complejos. Entre estas estructuras, podemos destacar el hipotálamo y la corteza prefrontal, dos áreas implicadas en diferentes procesos cognitivos como la memoria y la ingesta (**Carpenter-Hyland y Chandler, 2007; Phillips y cols., 2006**).

2.2 Sinapsis glutamatérgicas hipocampales y neurotransmisores

El L-glutamato (L-Glu) está presente en la mayoría de las sinapsis excitatorias del cerebro. Es el principal mediador de la información sensorial, motora, cognitiva y emocional y está especialmente implicado en la formación de la memoria, la neuroplasticidad y los procesos de aprendizaje hipocampales (**Danbolt, 2001; Malenka, 1994**). En este sentido, se ha visto que alteraciones en los niveles de L-Glu están asociados al desarrollo de enfermedades neurodegenerativas como la esclerosis lateral amiotrófica, la esclerosis múltiple, el Parkinson, la enfermedad de Huntington o la EA (**Beart y O'Shea, 2007; Danbolt, 2001; Malenka, 1994; Pitt y cols., 2000; Rothstein y cols., 1995**). Por lo tanto, es necesario conocer los mecanismos implicados en el correcto mantenimiento del equilibrio glutamatérgico en el hipocampo. Además, el L-Glu

es un sustrato para la síntesis de péptidos y proteínas o de moléculas como el glutatión e incluso se puede incorporar al ciclo de los ácidos tricarboxílicos y usarse como sustrato energético **(Danbolt, 2001; Gammelsaeter y cols., 2011; Han y Park, 2011**).

Por otro lado, el L-Glu está relacionado con el metabolismo del ácido γ-aminobutírico (GABA), principal neurotransmisor inhibitorio (**Bak y cols., 2006; Bolaños, 2016; Falkowska y cols., 2015**). Así, los astrocitos recaptan el L-Glu y el GABA liberados por las neuronas glutamatérgicas y GABAérgicas a través de los transportadores GLAST/GLT1 y GAT-A, respectivamente. A continuación, el GABA se transforma en L-Glu mediante carboxilación; el L-Glu capta amonio y se transforma en L-glutamina (L-Gln) por acción de la enzima glutamina sintetasa (GS). En este punto, la L-Gln puede liberarse a la sangre mediante los transportadores SNAT 3/5 o ser devuelta a las neuronas por los transportadores SNAT 1/2 (**Pochini y cols., 2014**), donde se convertirá nuevamente en L-Glu o GABA (Figura 2) (**Bolaños, 2016**).

Otro neurotransmisor relevante en el hipocampo es la D-serina (D-Ser). Este D-aminoácido procede de la isomerización de la L-serina (L-Ser) por acción de la serina racemasa (SR) (**Maugard y cols., 2021**). La L-Ser es un aminoácido no esencial, ya que puede sintetizarse a partir del 2-fosfoglicerato procedente de la glucólisis. Sin embargo, se trata de un metabolito fundamental en la biosíntesis de otros aminoácidos como la glicina (Gly) (**Furuya y cols., 2000; Maugard y cols., 2021**) o de lípidos como los esfingolípidos (**Furuya y cols., 2002**). Aunque la principal fuente de D-Ser en el hipocampo son los astrocitos (**Neame y cols., 2019**), también se ha encontrado síntesis neuronal y microglial (**Beltrán-Castillo y cols., 2018; Ding y cols., 2011**). La producción de D-Ser es esencial para el correcto funcionamiento de las sinapsis glutamatérgicas puesto que actúa como cofactor de los receptores ionotrópicos de glutamato de tipo NMDA (ácido N-metil-D-aspártico) (**Henneberger y cols., 2010**). De hecho, cambios en los niveles de D-Ser se han relacionado con alteraciones cognitivas asociadas a la demencia y la EA (**Henneberger y cols., 2010; Van-Horn y cols., 2013**).

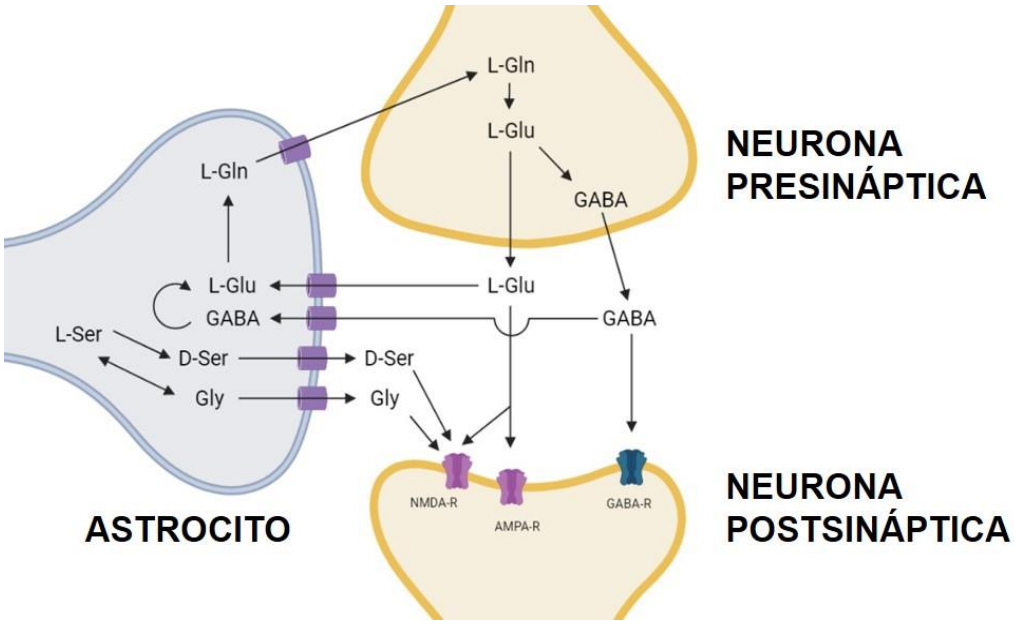


Figura 2. Neurotransmisión glutamatérgica entre la neurona y el astrocito*. La neurona presináptica glutamatérgica y GABAérgica, respectivamente, produce L-Glu a partir de L-Gln y GABA, a partir de L-Glu, que son liberados al espacio sináptico. Estos neurotransmisores son recaptados por los astrocitos donde se transforman nuevamente en L-Gln, que se lo devolverá a la neurona presináptica. Por otra parte, el astrocito también libera D-Ser y Gly, que actúan como cofactores del recetor de L-Glu NMDA. AMPA-R (receptor AMPA), NMDA-R (receptor NMDA), GABA-R (receptor GABA).*

2.3 Plasticidad sináptica: receptores y mecanismos

Las sinapsis químicas pueden verse alteradas por estímulos eléctricos y químicos, pudiendo aumentar o disminuir su actividad. Además, sabemos que los mecanismos responsables de estos cambios se producen en diferentes escalas de tiempo, pudiendo durar desde segundos hasta años. Al conjunto de dichas adaptaciones se las conoce como plasticidad sináptica (**Brager y cols., 2003; Buonomano y Merzenich, 1998; Deng y cols., 2011; Tamura y cols., 2011**). En el SNC hay algunos patrones de actividad que producen un aumento prolongado de la eficacia sináptica, como la potenciación a largo plazo o *long-term potentiation* (LTP), mientras que otros inducen una disminución duradera de la eficacia de las sinapsis, como la depresión a largo plazo o *long-term depression* (LTD) (**Grigoryan y cols., 2012; Lüscher y Huber, 2010; Pavlowsky y Alarcon, 2012**). El hecho de que estos patrones de actividad se mantengan a lo largo del tiempo en determinadas áreas como el hipocampo, permiten explicar los mecanismos implicados en la adquisición de tareas de aprendizaje, así como el almacenamiento de procesos de memoria a corto y

largo plazo (**Bowden y cols., 2012; Grigoryan y cols., 2012; Lüscher y Huber, 2010; Pavlowsky y Alarcon, 2012**).

La plasticidad sináptica hipocampal es un proceso regulado por numerosos mecanismos entre los que destaca la participación del sistema glutamatérgico. Para ello, el L-Glu interacciona con sus receptores ionotrópicos NMDA y AMPA (ácido α-amino-3-hidroxi-5-metilo-4-isoxazolpropiónico), así como con los receptores metabotrópicos NMDA 1 y 5 (**Balu and Coyle, 2011; Hamad y cols., 2011; Murakami y cols., 2006; Smith y cols., 2009**). Los receptores NMDA están implicados en el desarrollo sináptico y en la modulación de patrones de actividad como la LTP o la LTD (**Ge y cols., 2010; Malenka y Bear, 2004**). Dentro de los receptores NMDA, las subunidades 2A y 2B desempeñan un papel fundamental en los procesos de plasticidad. Ambas subunidades difieren tanto en su cinética como en su localización sináptica (**Kochlamazashvili y cols., 2010; Matta y cols., 2011; Swanger y Traynelis, 2018; Yashiro y Philpot, 2008**). Así, por ejemplo, la isoforma 2B tiene una mayor afinidad por la proteína dependiente de Ca^{2+}-calmodulina tipo II (CaMKII), otorgando a esta subunidad un papel esencial en la inducción de LTP y LTD (**Lisman y cols., 2002; Strack y cols., 2000; Strack y Colbran, 1998**).

Por otro lado, los receptores AMPA también son importantes para la inducción y el mantenimiento de la LTP y la LTD (**Clayton y cols., 2002; Malinow y Malenka, 2002**). Numerosos estudios electrofisiológicos muestran que la densidad de los receptores AMPA postsinápticos es determinante para la eficacia sináptica (**Lüscher y cols., 1999; Malenka y Nicoll, 1999; Malinow y Malenka, 2002**). El receptor AMPA presenta varias isoformas (1-4), siendo la subunidad 2 la necesaria para el ensamblaje y expresión del receptor, además de proporcionar una mayor resistencia a la excitotoxicidad disminuyendo la permeabilidad al calcio (**Boulter y cols., 1990; Lee y cols., 1998; Sans y cols., 2003**). Se ha demostrado que la densidad proteica de ambos receptores glutamatérgicos (AMPA y NMDA) en el hipocampo es esencial en el control de la plasticidad sináptica y, por tanto, sobre la capacidad cognitiva (**Adams y cols., 2001; Clayton y cols., 2002**).

Para que se desencadene la LTP es necesaria la entrada de Ca^{2+} a través de los receptores NMDA de la membrana postsináptica tras su unión al L-Glu (Figura 3). En condiciones normales, el receptor NMDA se encuentra bloqueado por iones Mg^{2+}. Cuando se produce un estímulo corto de alta frecuencia eléctrica, la despolarización genera el desplazamiento de los iones Mg^{2+}, dejando libre el canal del receptor NMDA y permitiendo la entrada de Ca^{2+}. Estos efectos son mediados por las subunidades NMDA-2A y NMDA-2B. El aumento de Ca^{2+} intracelular conduce a la activación de las quinasas dependientes de Ca^{2+} como la CAMKII, la proteína quinasa C (PKC) o la tirosina quinasa Fyn, las cuales son responsables de fosforilar los receptores AMPA. Dicha fosforilación induce la translocación de nuevos R-AMPA a la membrana, y una mayor sensibilidad a L-Glu (**Herring y Nicoll, 2016; Malenka, 1994; Yashiro y Philpot, 2008**). Por otra parte, si el estímulo es suficientemente intenso, el complejo Ca^{2+}-calmodulina puede activar a la proteína quinasa A (PKA), la cual se encarga de fosforilar el factor de transcripción CREBP (proteína de unión a elementos de respuesta a AMP cíclico). CREBP fosforilado viaja por el axón hasta llegar al soma, donde puede translocarse al núcleo y unirse a diferentes elementos de respuesta. Así, entre otros efectos, se induce la traducción de nuevos receptores AMPA que viajan de forma retrógrada hacia la membrana postsináptica. De esta manera, la potenciación se puede alargar en el tiempo hasta semanas **(Herring y Nicoll, 2016; Kandel, 2001**).

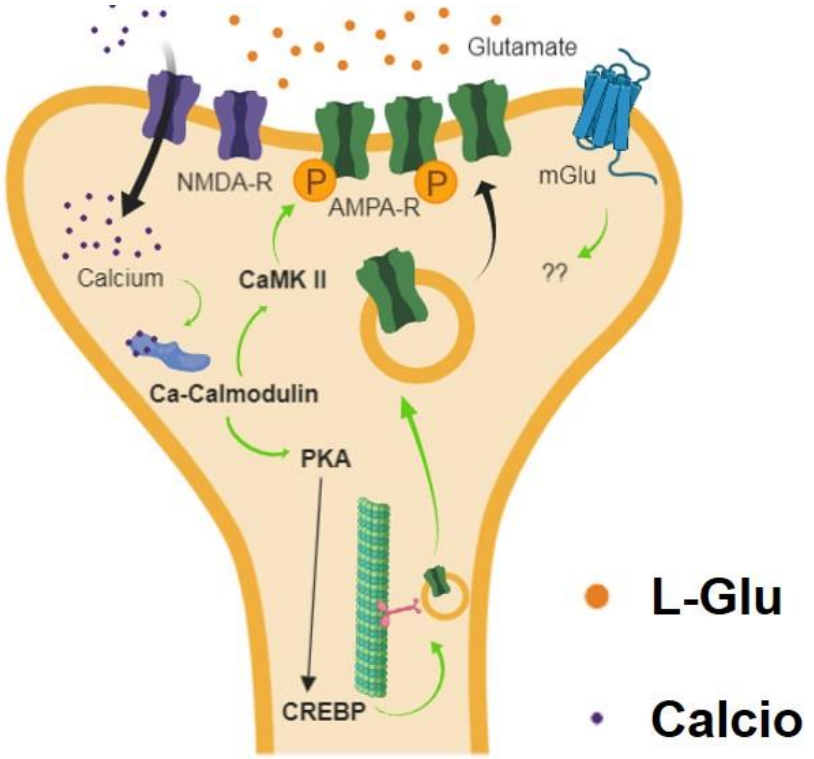


***Figura 3. Mecanismos de plasticidad sináptica: LTP**. La LTP se caracteriza por una entrada masiva y rápida de Ca^{2+} a la neurona postsináptica a través del receptor NMDA. En respuesta, se activa la Ca^{2+}-calmodulina que se une a la CaMK II promoviendo su actividad. La CaMK II fosforila a los receptores AMPA, aumentando su exocitosis a la membrana y la eficacia sináptica. Además, Ca^{2+}-calmodulina se une a la PKA, que mediante la fosforilación de CREBP, promueve la síntesis de nuevos receptores AMPA. R-AMPA (receptor AMPA), CAMK II (quinasa dependiente de Ca^{2+}-calmodulina II), CREBP (proteína de unión a los elementos de respuesta de AMP cíclico), L-Glu (L-glutamato), RGlum (receptor metabotrópico de glutamato), R-NMDA (receptor NMDA) y PKA (proteína quinasa A).*

Por otro lado, la LTD también requiere de la activación de los receptores NMDA postsinápticos, aunque este mecanismo se traduce en una disminución de la señal sináptica (Figura 4). Si el estímulo es de baja frecuencia y prolongado en el tiempo, la entrada de Ca^{2+} lenta y progresiva conduce a la activación de fosfatasas como la proteína fosfatasa 2B (PP2B), conocida también como calcineurina. PP2B puede, a su vez, desfosforilar la proteína fosfatasa 1 (PP1). Ambas fosfatasas desfosforilan los receptores AMPA, induciendo su endocitosis y una disminución de su conductancia (**Collingridge y cols., 2010; Kandel, 2001**). Además, PP2B también desforila a la proteína quinasa B (PKB/Akt), dando lugar a la activación de la proteína quinasa de la glucógeno sintasa 3β (GSK-3β), la cual estimula la LTD mediante la disminución de los R-AMPA postsinápticos (**Collingridge y cols., 2010**).

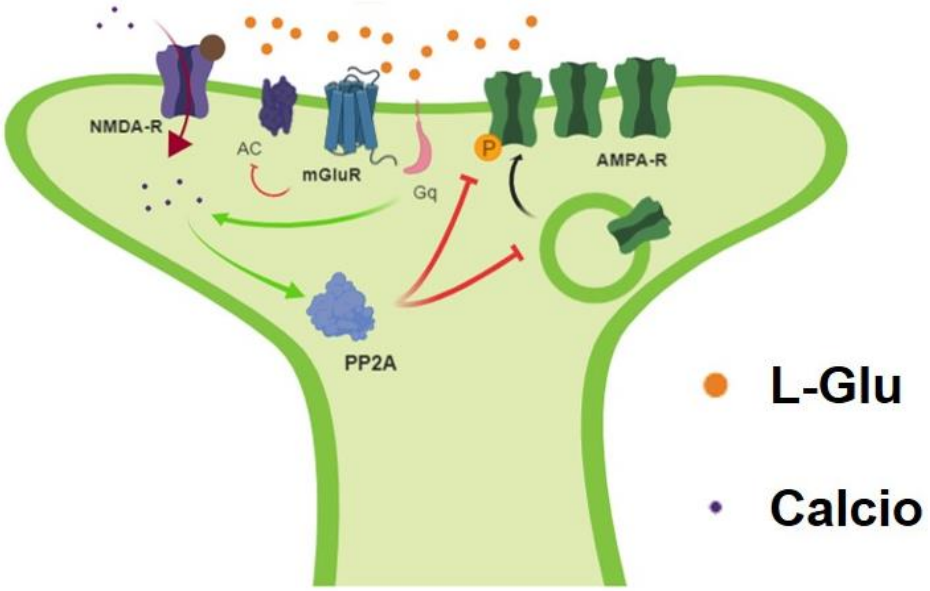


***Figura 4. Mecanismos de plasticidad sináptica: LTD**. La LTD se induce por una entrada lenta y prolongada de calcio a la neurona postsináptica a través del receptor NMDA. En este caso se activan diferentes fosfatasas como la PP2A, que desfosforila a los receptores AMPA, induciendo su endocitosis y, por tanto, disminuyendo la eficacia sináptica. Parece que también podrían estar involucrados los mGluR. R-AMPA (receptor AMPA), L-Glu (L-glutamato), RGlum (receptor metabotrópico de glutamato), R-NMDA (receptor NMDA) y PP2A (proteína fosfatasa 2A).*

2.4 Aprendizaje e ingesta

Desde hace décadas se sabe que el hipotálamo es el centro regulador de la ingesta y del peso corporal. Sin embargo, el control de la conducta alimentaria depende también de otras regiones cerebrales como el hipocampo y la corteza prefrontal. (**Zald, 2009**). Numerosos estudios sugieren que los mecanismos de aprendizaje dependientes del hipocampo contribuyen activamente al control de la conducta alimentaria. (**Davidson y cols., 2007; Kanoski y cols., 2007; Stranahan y Mattson, 2008**). En este sentido, el hipocampo, aparte de codificar la memoria espacial y la formación/recuperación de recuerdos, también es necesario para relacionar estímulos internos con sus comportamientos asociados (**Richard, 2006**). De esta forma, el hipocampo media el aprendizaje necesario para identificar las señales de apetito y saciedad para tomar la decisión de comer o no comer (**Davidson et al., 2005**), todo a través de la conjunción entre señales cerebrales y hormonas como la leptina y los glucocorticoides (**Dallman, 2010; Lathe, 2001**).

3. HIPOTÁLAMO

3.1 Estructura y funciones generales

El hipotálamo es una estructura situada bajo el tálamo, que forma parte de la pared lateral del tercer ventrículo. Se extiende desde la zona superior del quiasma óptico (área supraóptica), área preóptica, área tuberal, hasta los márgenes posteriores de los cuerpos mamilares (Figura 5). El área supraóptica o hipotálamo anterior, situada en el tronco cerebral, contiene el núcleo supraquiasmático y el quiasma óptico, y desempeña un papel crucial en la regulación neuroendocrina. Por otro lado, el hipotálamo anterior comprende dorsalmente los núcleos paraventricular y preóptico con funciones endocrinas, y ventralmente el área hipotalámica anterior y el núcleo preóptico lateral, que participan en funciones de regulación interna como la temperatura corporal. El área tuberal o hipotálamo medial está formada por los núcleos arqueado, dorsomedial y ventromedial, y el hipotálamo lateral (ventral). Todas estas áreas están implicadas en la regulación de las emociones, la función hipofisaria, el apetito y la saciedad. Por último, el área mamilar o hipotálamo posterior engloba, entre otros, la parte caudal del núcleo arqueado y está fuertemente implicada en la memoria (**Flament-Durand, 1980**).

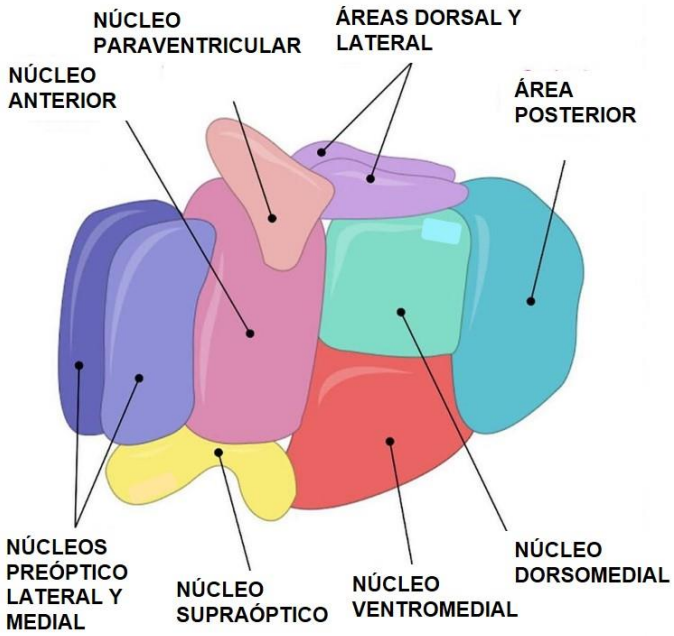


Figura 5. Núcleos hipotalámicos. *Representación gráfica del conjunto de núcleos que conforman el hipotálamo.*

3.2 Áreas y mediadores involucrados en la regulación de la ingesta

Numerosos estudios han demostrado la implicación de los núcleos arqueado, ventromedial, dorsomedial, paraventricular y lateral en el control de la homeostasis energética, ya que la lesión o estimulación de estas áreas hipotalámicas genera cambios en la ingesta y el gasto energético (**Roger y cols., 2021; Timper y Brüning, 2017; Woods y cols., 2000**). El hipotálamo posee una barrera hematoencefálica más laxa, lo que le permite estar en contacto estrecho con los nutrientes (glucosa, grasas, aminoácidos, etc.) y hormonas (insulina, leptina, glucocorticoides, etc.) que viajan en la sangre. Así, en función de los niveles de estos compuestos, puede liberar péptidos saciantes o anorexigénico o péptidos que promuevan la ingesta u orexigénicos. De esta manera, el equilibrio entre estos mediadores es responsable del adecuado control de la ingesta y del peso corporal (**Kalra y cols., 1999; Sohn, 2015; Woods y cols., 2000**).

Entre los neuropéptidos orexigénicos destaca el neuropéptido Y (*Neuropeptide Y*, NPY), cuya liberación aumenta durante el ayuno y disminuye tras la ingesta. Se ha visto que la administración de NPY induce hiperfagia y aumento de peso (**Stanley y cols., 1985**), lo que se acompaña de una disminución de los niveles de leptina y del gasto energético, reduciéndose además la termogénesis en el tejido adiposo marrón (TAM) y la actividad simpática (**Billington y cols., 1991; Izawa y cols., 2022; Zhang y cols., 2014**). Además, existen otros sistemas alternativos al NPY, como el péptido relacionado con el Agouti (*Agouti Related Protein*, AgRP) y la hormona concentradora de melanina (*Melanine Concentrating Hormone*, MCH) (Figura 6). La expresión de ambos péptidos está inhibida por la leptina (**Ludwig y cols., 1998; Mizuno y Mobbs, 1999**).

Por otro lado, uno de los principales sistemas anorexígenos son los péptidos derivados de la proopiomelanocortina (*Proopiomelanocortin*, POMC), que incluyen la adrenocorticotropina (*Adrenocorticotropin Hormone*, ACTH), la hormona estimulante de los melanocitos alfa (α-*Melanostimulant Hormone*, αMSH) y la β-endorfina (**Dutia y cols., 2012; Huszar y cols., 1997**) (**Fan y cols., 1997; Huszar y cols., 1997**) (Figura 6). Finalmente, la hormona liberadora de corticotropina (*Corticotropin Releasing Hormone*, CRH) estimula la liberación de

ACTH, produciendo anorexia e inhibiendo la ingesta inducida por el NPY (**Kalra y cols., 1999**).

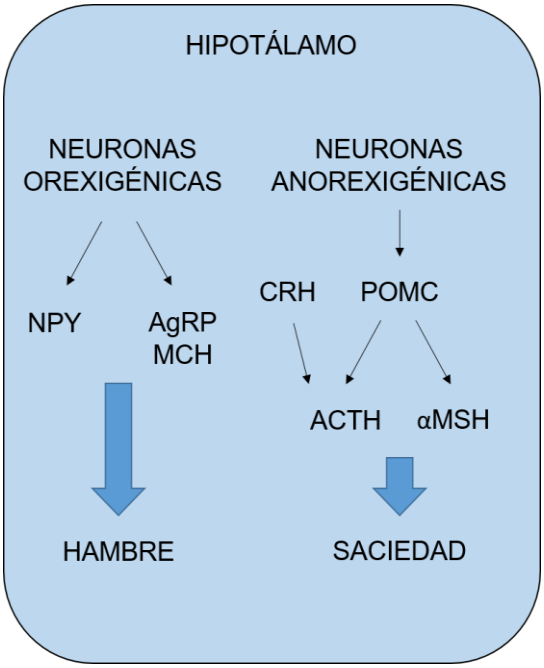


Figura 6. Mediadores hipotalámicos implicados en la ingesta. *Dentro del hipotálamo hay dos grupos de neuronas: orexigénicas y anorexigénicas. Su actividad conjunta regula la ingesta. NPY (neuropéptido Y), AgRP (péptido relacionado con Agoutí), MCH (hormona concentrante de la melanina), CRH (hormona liberadora de la corticotropina), POMC (proopiomelanocortina), ACTH (hormona adrenocorticotrópica) y αMSH (hormona estimulante de melanocitos α).*

4. TEJIDO ADIPOSO

4.1 Tipos de tejido adiposo

Histológicamente, existen dos tipos de tejido adiposo, el tejido adiposo blanco (TAB) y el tejido adiposo marrón (TAM). El TAB presenta adipocitos univesiculares con gran capacidad de almacenamiento de energía en forma de triglicéridos. En el TAM, los adipocitos son multivesiculares y son ricos en mitocondrias, necesarias para promover la termogénesis.

El TAB representa aproximadamente el 20% del peso corporal de un adulto y se diferencia entre TAB subcutáneo (TAB-Sc) y TAB visceral (TAB-Vis) (**Ibrahim, 2010**). El TAB-Sc se sitúa bajo la dermis y en la zona inguinal posterior y anterior, mientras que el TAB-Vis se localiza dentro de la cavidad abdominal y, según el órgano que rodea, se habla de perigonadal (TAB-Pg), perirrenal (TAB-Per), omental (TAB-Om), perivascular (TAB-Pv) o mesentérico (TAB-Ms) (Figura 7). El TAB es un tejido formado mayoritariamente por adipocitos maduros,

rodeados de tejido conectivo altamente inervado y vascularizado, y por el estroma vascular, que consta de fibroblastos, macrófagos, preadipocitos (precursores de adipocitos maduros), células madre mesenquimales y otros tipos celulares (**Ahima, 2006**). La función principal del TAB es el almacenamiento de energía durante periodos postprandiales, vinculados a los niveles plasmáticos elevados de insulina, glucosa y lípidos (**Vázquez-Vela y cols., 2008**). Además, el TAB está inervado por el sistema nervioso autónomo, que en situaciones de ayuno estimula la lipolisis (**Fliers y cols., 2003; Kreier y cols., 2002**).

4.2 Diferencias entre el TAB subcutáneo y visceral

Los TAB-Sc y -Vis son tejidos que presentan diferencias metabólicas y endocrinas. Desde un punto de vista metabólico se ha visto que el TAB-Vis presenta una mayor expresión de genes relacionados con la lipogénesis por lo que tiene una tendencia natural a hipertrofiarse (**Ronquillo y cols., 2019**). Además, este tejido adiposo es capaz de captar más glucosa y liberar más ácidos grasos libres al plasma, sobre todo en respuesta a insulina y catecolaminas, respectivamente (**Ibrahim, 2010**). Por el contrario, el TAB-Sc es el que mayoritariamente capta el exceso de grasa que ingerimos con la dieta, presenta adipocitos más pequeños y una expresión equilibrada de enzimas lipogénicas y lipolíticas (**Arner, 1997; Freedland, 2004**). Esto convierte al TAB-Sc en un tejido muy plástico y, por tanto, adaptativo a los cambios en la ingesta energética.

En relación con la función endocrina del TAB diversos autores han demostrado que el TAB-Sc es la principal fuente de leptina, mientras que el TAB-Vis libera más adiponectina al plasma (**Freedland, 2004; Wajchenberg, 2000**). Por otra parte, el TAB-Vis está más infiltrado de células inflamatorias. Así, durante la obesidad el TAB-Vis es el principal productor de TNF-α, IL-6 y proteína 1 quimioatrayente de monocitos (MCP-1) (**Pou y cols., 2007; Spoto y cols., 2014; Yudkin y cols., 2005**). Esto, unido a que este tejido presenta adipocitos más grandes, le hace más susceptible a desarrollar resistencia a insulina (**Frayn, 2000; Marette, 2003**). El TAB-Sc, en cambio, es más resistente a inflamarse durante la obesidad, además de que disminuyen los marcadores inflamatorios antes que en el -Vis frente a las terapias anti-obesidad (**Spoto y cols., 2014**).

Con respecto a la respuesta a hormonas, el TAB de las mamas y las nalgas (subcutáneo) es muy sensible a los estrógenos. Por el contrario, tejido adiposo localizado en la cavidad abdominal es más sensible a los glucocorticoides y andrógenos (**del Mar Romero y cols., 2009; Tchkonia y cols., 2002**). Esta puede ser la razón del dimorfismo sexual en cuanto a la distribución corporal de cada tejido adiposo (**Esteve Ràfols, 2014**).

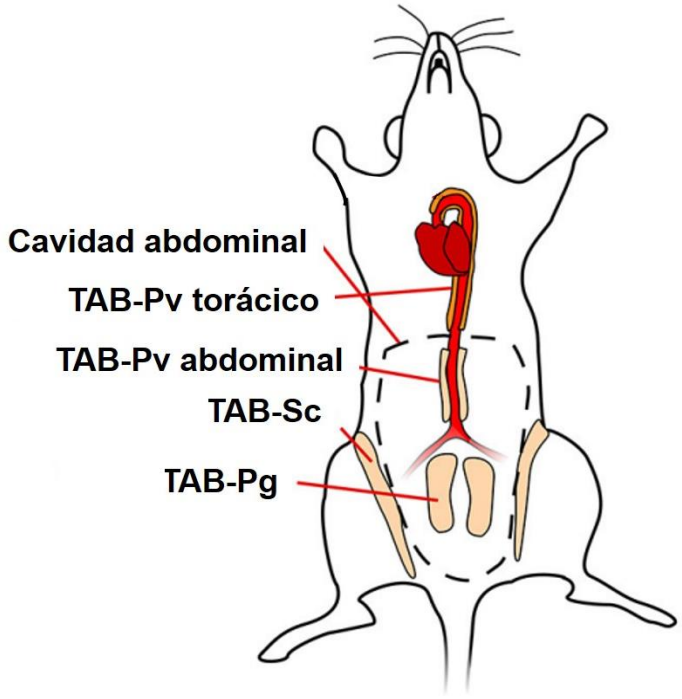


Figura 7. Tipos de tejido adiposo según su localización anatómica. *En la figura aparecen el TAB-Pv (perivascular) de la aorta, el TAB-Sc (subcutáneo) y el TAB-Pg (perigonadal), rodeando las gónadas, como ejemplo de TAB-Vis (visceral).*

4.3 Expansibilidad del tejido adiposo blanco

Ante una situación de desequilibrio energético, los adipocitos del TAB tienen la capacidad de adaptarse mediante el aumento de su tamaño (hipertrofia) y de su número (hiperplasia). La hiperplasia debe ir acompañada de un aumento en la vascularización para evitar la hipoxia celular y permitir una correcta perfusión (**Ledoux y cols., 2008**). De esta manera, una hipertrofia del TAB junto a una angiogénesis insuficiente, desencadena la activación de mecanismos pro-inflamatorios capaces de deteriorar la función endocrina del TAB, promoviendo la aparición de resistencia a insulina y la acumulación ectópica de lípidos, entre otros (**Goossens, 2017; Muñoz-Garach y cols., 2016**). Por el contrario, una expansión equilibrada favorece una adecuada función endocrina, lo que se

conoce como individuos obesos metabólicamente sanos (**Blüher, 2020**). Existe también el caso de la pérdida de plasticidad del TAB en individuos con peso corporal normal, dando lugar a individuos delgados metabólicamente obesos (**Virtue y Vidal-Puig, 2008**).

La hiperplasia del TAB tiene su origen en las células mesenquimales del estroma vascular. En una situación de equilibrio energético positivo, se secretan factores pro-adipogénicos, como las proteínas de la familia de las proteínas morfogénicas de hueso (BMPs) (**Gupta y cols., 2010; Huang y cols., 2009**). Las BMPs inducen la diferenciación de las células mesenquimales del TAB a adipocitos mediante la activación de una cascada de factores de transcripción específicos. Entre los factores de transcripción más relevantes en este proceso destacan el receptor de activadores de la proliferación de peroxisomas γ (PPARγ) y la proteína potenciadora de unión a CCAAT α (C/EBPα) **(Farmer, 2006; Rosen y MacDougald, 2006; Siersbæk y cols., 2012**).

4.4 El tejido adiposo blanco como órgano endocrino

El TAB funciona como un órgano secretor capaz de liberar numerosos factores endocrinos y paracrinos conocidos como adipoquinas, que actúan a nivel central y periférico participando en el control del metabolismo y de la ingesta (**Galic y cols., 2010; Trujillo y Scherer, 2006**). La mayoría de las adipoquinas son producidas por los adipocitos, pero algunas como la resistina o la IL-6 son secretadas por los macrófagos residentes en el tejido adiposo, donde actúan de forma paracrina controlando el metabolismo adipocitario. Las adipoquinas producidas exclusivamente por el TAB incluyen la adiponectina, la **leptina** o el factor de necrosis tumoral (TNF-α), además de los ácidos grasos no esterificados (NEFA).

5. EJE ADIPOSO-SISTEMA NERVIOSO CENTRAL: PAPEL DE LA LEPTINA

El equilibrio energético en el organismo es el resultado de un equilibrio entre la ingesta, el gasto de energía y el almacenamiento de energía. La regulación de estos parámetros se lleva a cabo mediante la generación de señales que viajan desde el TAB al SNC y viceversa, creándose un eje adiposo-SNC (**Schulz y cols., 2010; Shimizu y Mori, 2005**). Entre todas estas señales, la leptina tiene una importancia esencial, puesto que no sólo se secreta en

proporción directa a la cantidad de grasa corporal, sino que además tiene un impacto directo sobre todos los componentes de eje: presenta un efecto autocrino y efectos endocrinos sobre todos los tejidos/órganos, incluido el SNC.

5.1 La leptina como hormona

La leptina es un péptido de 167 aminoácidos (16 kDa) producido por el gen *Ob*. Se sintetiza principalmente en el TAB, aunque también hay producción de leptina en el estómago, la placenta, el cerebro y el músculo esquelético **(Ahima y Flier, 2000**).

Los niveles de leptina plasmática son directamente proporcionales a la cantidad total de TA. De hecho, los individuos obesos presentan mayores concentraciones de leptina en plasma que los no obesos (**Farr y cols., 2015**). Por otra parte, la secreción de esta hormona varía de acuerdo al ritmo circadiano relacionado con la ingesta, y con los ritmos de luz-oscuridad **(Zhang y cols., 1994**; **Wajchenberg, 2000**). De este modo, los niveles de leptina en humanos aumentan durante el día, mientras que, en los roedores son mayores durante la noche. La producción de leptina también está estimulada por otros factores como la insulina, el TNF-α, los glucocorticoides o la interleuquina-1 (IL-1) (**Havel, 2001**; **Bornstein y cols., 1997; Simón y Del Barrio, 2002**). Así mismo, la activación simpática del tejido adiposo durante el ayuno inhibe la expresión de esta hormona (**Deng y cols., 1997**).

5.2 El receptor de leptina y su señalización

El receptor de leptina es un receptor perteneciente a la familia de los receptores de citoquinas de clase I. Esta familia incluye receptores para interleuquinas como la IL-4 y la IL-6, el factor estimulante de granulocitos, la hormona del crecimiento, la prolactina y la eritropoyetina (**Bazan, 1989**).

Se han descrito 6 isoformas del receptor, ObRa, ObRb, ObRc, ObRd, ObRe y ObRf (Figura 8) (**Tartaglia y cols., 1995**), las cuales forman homodímeros que se activan por cambios conformacionales inducidos por la unión de la leptina (**Devos y cols., 1997**). Todas ellas, excepto el Ob-Re, son receptores de membrana con un dominio transmembrana común y un dominio intracelular, característico de cada isoforma (**Bjørbaek y cols., 1997**). Así,

podemos diferenciar entre las isoformas cortas del receptor de leptina (ObRa, ObRc, ObRd y ObRf) y la isoforma larga (ObRb). Finalmente, existe una forma soluble (ObRe), con gran afinidad por la leptina plasmática, regulando así su biodisponibilidad (**Heaney y Golde, 1993**). La isoforma ObRa se localiza principalmente en el plexo coroideo, los microvasos cerebrales y las meninges (**Devos y cols., 1997; Elmquist y cols., 1998**) y parece estar implicada en el transporte de leptina al SNC. La isoforma ObRb se expresa en tejidos periféricos, como el TA (**Kutoh y cols., 1998**), y en el SNC en áreas como el hipotálamo, el hipocampo, el cerebelo y el tálamo (**Elmquist y cols., 1998; Schwartz y cols., 1996; Tartaglia y cols., 1995**). Esta isoforma es la responsable de las acciones de la leptina, ya que presenta un dominio intracelular específico acoplado a la señalización del receptor de leptina.

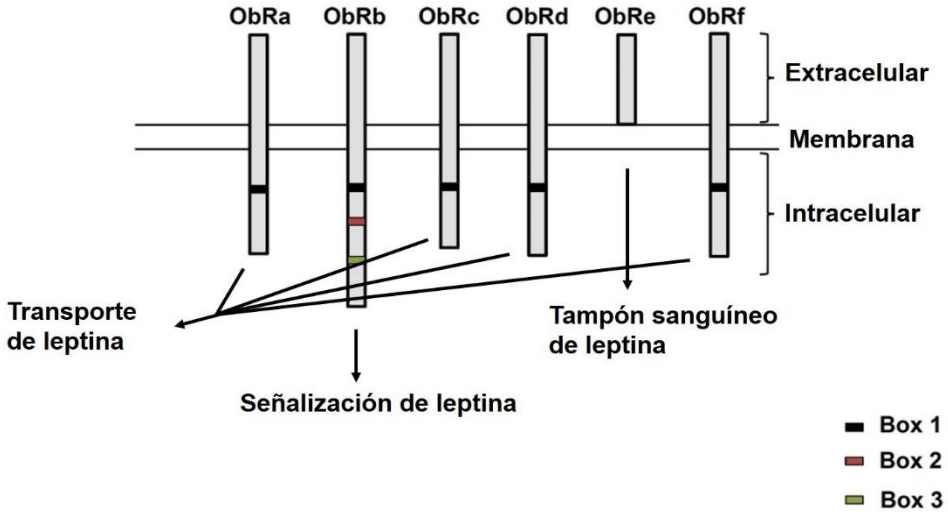


Figura 8. Estructura y funciones del receptor de leptina (*modificado de* ***Thon y cols., 2016).*** *ObRa, ObRc, ObRd y ObRf corresponden a las isoformas cortas; ObRb, isoforma larga y ObRe isoforma soluble del receptor de leptina*.

Como se ha mencionado anteriormente, el receptor ObRb es el responsable de la señalización de leptina. Se han identificado diferentes vías de señalización intracelular acopladas al ObRb, siendo la ruta de las JAK-STAT (*Janus Tyrosine kinase-Signal transducer and activator of transcription*) la vía de señalización principal (Figura 9) (**Bjørbaek y cols., 1997; Vaisse y cols., 1996**). La unión de la leptina a ObRb induce un cambio conformacional que permite el reclutamiento y posterior autofosforilación de JAK2/3. A continuación, las JAK2/3 fosforilan las Tyr^{985}, Tyr^{1077} y Tyr^{1138} del receptor ObRb. Cada uno de estos

residuos de tirosina activa una vía de señalización específica implicada en las diferentes funciones fisiológicas de la leptina. Así, Tyr^{985} activa la vía de las proteínas quinasas dependientes de mitógenos (MAPK), responsable de los mecanismos de retroalimentación negativa de la misma vía; Tyr^{1077} activa STAT5, implicada en los efectos de la leptina en la reproducción (**Myers y cols., 2008**). Finalmente, Tyr^{1138} promueve la activación de STAT3, responsable de los efectos neuroendocrinos de la leptina y sobre la homeostasis energética. La fosforilación de STAT3 (pSTAT3) permite su separación del receptor formando homo- y heterodímeros capaces de entrar en el núcleo, donde actúan como reguladores transcripcionales de diversos factores, induciendo la expresión de proteínas involucradas en los efectos metabólicos de la leptina. Algunas de las proteínas inducidas tras la activación del ObRb son la proteína tirosina fosfatasa 1B (PTP1B) y el supresor de la señalización de citoquinas 3 (SOCS3) (Figura 9). Ambas proteínas actúan como reguladores negativos, bloqueando la activación de las JAKs. De hecho, se ha demostrado que tanto la falta de SOCS3 como de PTPT1B aumentan la sensibilidad a la leptina y a la insulina (**Bjørbaek y Kahn, 2004; Cheng y cols., 2002**).

Además del eje JAK2-STAT3, la leptina es capaz de activar la vía de los inosítidos fosforilados PI3K-mTOR-Akt, en común con la insulina (Figura 9) (**Frühbeck, 2006**). Así, la interacción de la leptina a su receptor ObRb induce la fosforilación del sustrato del receptor de insulina 1/2 (IRS1/2), permitiendo su unión a la quinasa activada por PIP_3 (PI3K), que a través de la subunidad p85, fosforila a numerosos sustratos, incluyendo la serina/treonina quinasa proteína quinasa B (PKB) o Akt. Akt está implicada en la regulación de múltiples procesos fisiológicos, como el metabolismo de la glucosa, la proliferación celular, la apoptosis, los fenómenos de transcripción y la migración celular (**Manning y Toker, 2017**). Por otra parte, la leptina también es capaz de activar a la quinasa dependiente de AMP (AMPK) (Figura 9). La activación de la AMPK es responsable del aumento del gasto energético inducido por la leptina. Sin embargo no está clara la conexión entre el ObRb y la AMPK (**Ahima y cols., 2000; Zhang y cols., 2005**).

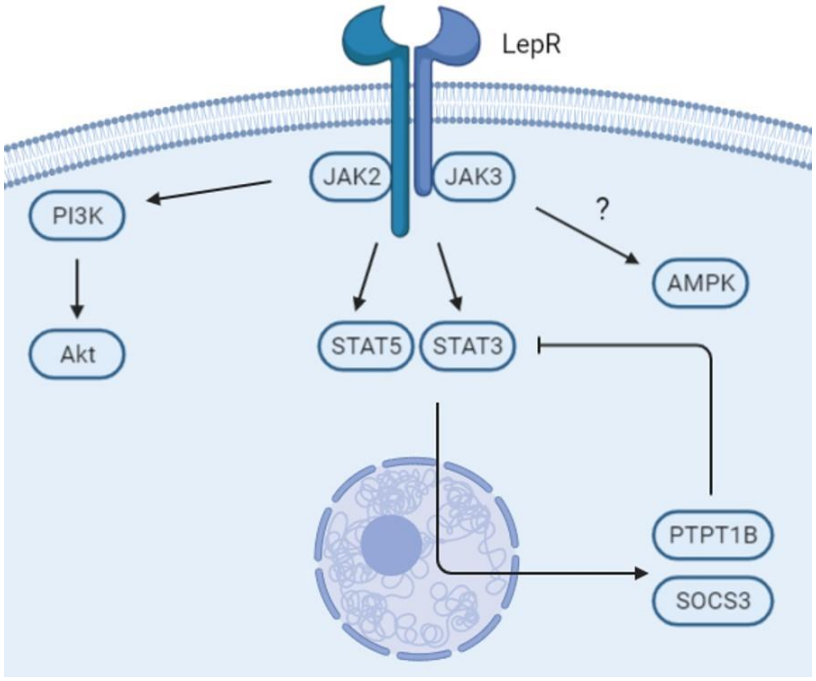


Figura 9. Señalización acoplada al receptor de leptina ObRb. *El receptor de leptina está acoplado a las quinasas JAK2 y 3. La unión de la leptina a ObRb induce la autofosforilación de las JAKs y la posterior fosforilación de otras proteínas como la STAT3 y STAT5. Por otra parte, la unión de la leptina a ObRb también activa la vía PI3K-Akt y AMPK. La activación de estas vías de señalización induce la expresión de PTPT1B y de SOCS3 como mecanismos de regulación negativa del receptor. AMPK (quinasa activada por AMP), JAK (Janus kinase), PI3K (quinasa activada por fosfoinositoles), PTPT1B (proteína fosfatasa de tirosinas fosforiladas 1B), SOCS3 (supresor de la señalización de citoquinas 3) y STAT (Signal transducer and activator of transcription).*

5.3 Efectos de la leptina sobre los componentes del eje adiposo-SNC

5.3.1 Efecto autocrino

Los receptores de leptina se expresan en la mayoría de los tejidos. Sin embargo, el en TAB los niveles de expresión de receptores de leptina (ARNm y proteína) son bajos (**Bornstein y cols., 2000; Ramsay y Richards, 2004**). A pesar de esto, en el TAB se han identificado la isoforma larga ObRb y las isoformas cortas, lo que sugiere que la leptina podría tener efectos paracrinos y autocrinos (**Huan y cols., 2003**).

Los estudios sobre el efecto de la leptina en el TAB son limitados y la mayoría son ensayos *in vitro* y *ex vivo* llevados a cabo en cultivos de adipocitos. Los estudios *in vitro* han demostrado que la leptina puede influir directamente en el metabolismo lipídico. Diversos estudios han demostrado que la exposición de los adipocitos a la leptina inhibe la síntesis de ácidos grasos (**Wang y cols., 2008**), además de promover su oxidación a través de un aumento del PGC -1

(**Kakuma y cols., 2000; Sáinz y cols., 2009**). Por otra parte, también se ha visto que la leptina aumenta la entrada de glucosa a los adipocitos (**Harris, 2014**), sobre todo si se han pretratado con insulina, indicando que existen mecanismos de señalización comunes (**Müller y cols., 1997**) (Figura 10).

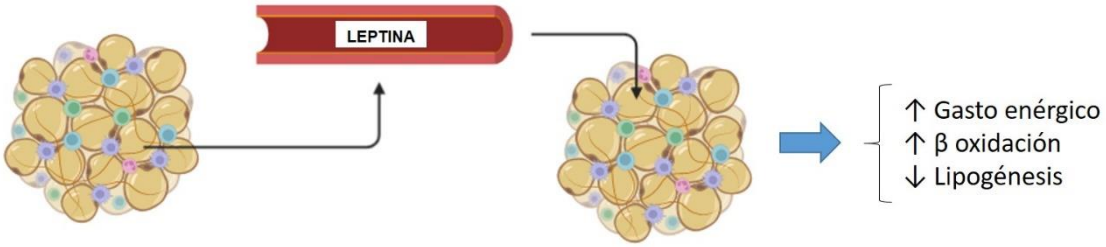


Figura 10. Efectos de la leptina sobre el TAB.

En cuanto a la resistencia a leptina, algunos autores han propuesto que el TAB también puede desarrollar resistencia a esta hormona (**Stucchi y cols., 2011**), lo que explicaría, en parte, por qué en animales obesos hiperleptinémicos, o no existen cambios o aumenta la masa total de TAB (**Wang y cols., 2000**). Estudios llevados a cabo con modelos animales de obesidad inducida por la dieta han demostrado que la administración de leptina intraperitoneal no estimula la activación de la vía JAK-STAT3 (**Guzmán-Ruiz y cols., 2012**). La resistencia a la leptina en TAB, además se ha asociado a una elevación de los niveles de SOCS1 y 3, además de una supresión de la expresión de ObRb (**Wang y cols., 2005**).

En relación con la expansibilidad del TAB, se ha observado que bajas concentraciones de leptina inhiben la proliferación de preadipocitos en cultivo, lo que sugiere que la leptina limitaría el crecimiento del TAB cuando la ingesta es normocalórica (**Wagoner y cols., 2006**). Sin embargo, cuando los niveles de leptina son elevados se ha observado la activación de las MAPKs y de la STAT3 **(Machinal-Quélin y cols., 2002**), que podrían inducir la proliferación de preadipocitos y un crecimiento acelerado del tejido capaz de afectar a su perfusión y activar procesos inflamatorios (**Bogacka y cols., 2004; Ramsay, 2005**). Aun así, hacen falta más estudios sobre el efecto autocrino/paracrino de la leptina tanto en un contexto fisiológico como en obesidad.

5.3.2 Efectos sobre el hipotálamo

La leptina es una hormona esencial en el control de la ingesta, el peso corporal y el gasto energético (Figura 11). El papel de la leptina en la regulación de la homeostasis energética se demostró al observar que la administración crónica de leptina a ratones *ob/ob* (carentes del gen de leptina) disminuía la ingesta y el peso corporal (**Halaas y cols., 1995**).

Tras la ingesta, aumentan los niveles plasmáticos de leptina y ésta se une al Ob-Ra de la barrera hematoencefálica, accediendo al SNC mediante un mecanismo de transporte saturable (**Banks y cols., 1996**). En el hipotálamo, la leptina se une al receptor Ob-Rb del núcleo arqueado, disminuyendo la expresión de péptidos orexígenos como el NPY y el AgRP y aumentando la expresión de péptidos anorexígenos como el POMC y el CART. Asimismo, en el núcleo ventromedial, estimula la expresión de neurotrofinas como el factor neurotrófico derivado del cerebro (BDNF) implicado en la sensación de saciedad (**Elmquist y cols., 1998; Schwartz y cols., 1996**). Se ha visto que los cambios en el patrón de expresión del BDNF, junto a la hiperleptinemia y la resistencia central a la leptina que se producen en determinadas situaciones de balance energético positivo, pueden contribuir a las alteraciones de la plasticidad neuronal hipotalámica y a la aparición de déficits cognitivos asociados a la ingesta (**Dickson y Chowen, 2020; Hoane y cols., 2011; Timper y Brüning, 2017**) .

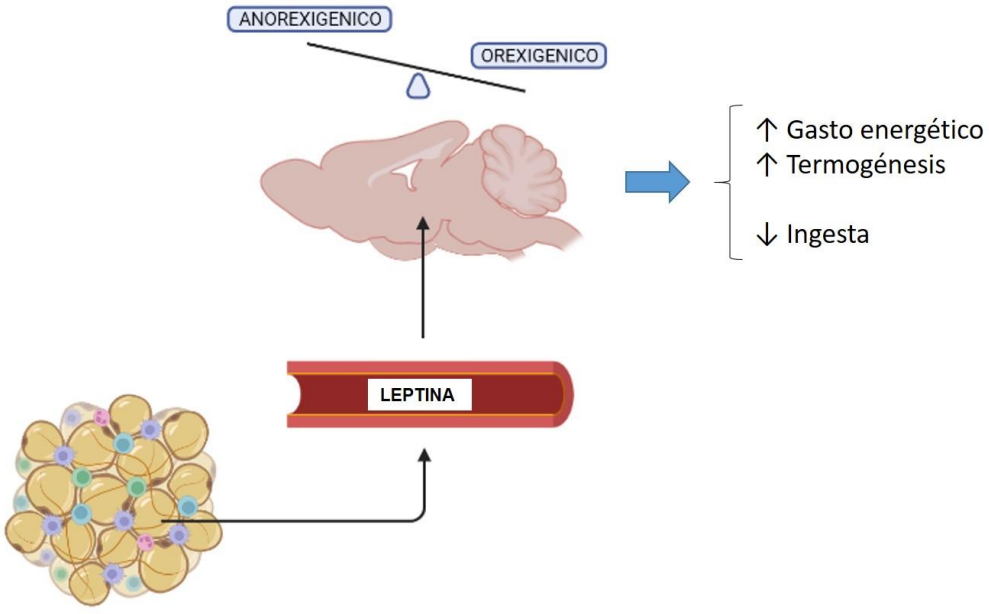


Figura 11. Efectos de la leptina sobre la regulación de la ingesta en el hipotálamo.

Aunque el receptor de leptina ObRb en el hipotálamo está principalmente acoplado a la activación de PI3K y Ras, la vía JAK-STAT desempeña un papel esencial en las funciones metabólicas de la leptina, puesto que es esta vía la involucrada en aumentar la expresión de POMC y reprimir la expresión de NPY/AgRP (**Harrold y cols., 1998; Myers y cols., 2008**). Además, parece que la leptina también ejerce parte de sus efectos hipotalámicos mediante la inhibición de la CPT1a y la AMPK, procesos necesarios para el control de la ingesta y el peso corporal (**Minokoshi y cols., 2004**). La inhibición de la AMPK, a diferencia de otros tejidos, se debe a que la leptina en el hipotálamo activa la calmodulina quinasa y la quinasa p70S6, las cuales al fosforilar a la AMPK disminuyen su actividad (**Dagon y cols., 2012; Dzamko y cols., 2010**).

5.3.3 Efectos sobre el hipocampo

En el hipocampo se han identificado las isoformas ObRa y ObRb del receptor de leptina, especialmente en GD y CA1 (**Schaab y cols., 2012**). Además, numerosos estudios han demostrado la implicación de la leptina en la regulación de la transmisión sináptica y la plasticidad neuronal en el este área (Figura 12) (**Harvey, 2007; Van Doorn y cols., 2017**). En este sentido, se ha observado que el ratón *db/db* presenta alteraciones en los dos patrones de plasticidad sináptica hipocampal, la LTP y la LTD. Dichas alteraciones se correlacionan con déficits en la realización de tareas de aprendizaje espacial como el laberinto de agua de Morris o el laberinto radial de 8 brazos (**Li y cols., 2002; Valladolid-Acebes y cols., 2011**). Otros autores han demostrado que la administración directa de leptina en GD o en CA1 aumenta la magnitud de la LTP (**Wayner y cols., 2004**) y mejora la memoria espacial de los animales (**Farr y cols., 2006**). En esta línea, estudios recientes han puesto de manifiesto que la leptina promueve la plasticidad sináptica dependiente de NMDA en el hipocampo (**Harvey, 2007b; McGregor y Harvey, 2018; Oomura y cols., 2006**). Por otra parte, ante situaciones de hiperexcitabilidad, como cuando se sobre-estimula a neuronas piramidales en CA1, la leptina es capaz de reducir la respuesta sináptica. Parece que este proceso es una forma especial de LTD dependiente de la síntesis *de novo* del receptor NMDA (**Durakoglugil y cols., 2005; Moult y Harvey, 2009**). Estos mecanismos inducidos por leptina reducen la hiperexcitabilidad y mantienen una eficacia sináptica adecuada. Por último, la

leptina aumenta la densidad y la movilidad de las espinas dendríticas en las sinapsis excitatorias del hipocampo (**O'Malley y cols., 2007**), lo que demuestra que la leptina participa en la regulación de la eficacia sináptica excitatoria. No obstante, todavía son necesarios más estudios para establecer los mecanismos exactos por los que la leptina regula la estructura y las funciones del hipocampo.

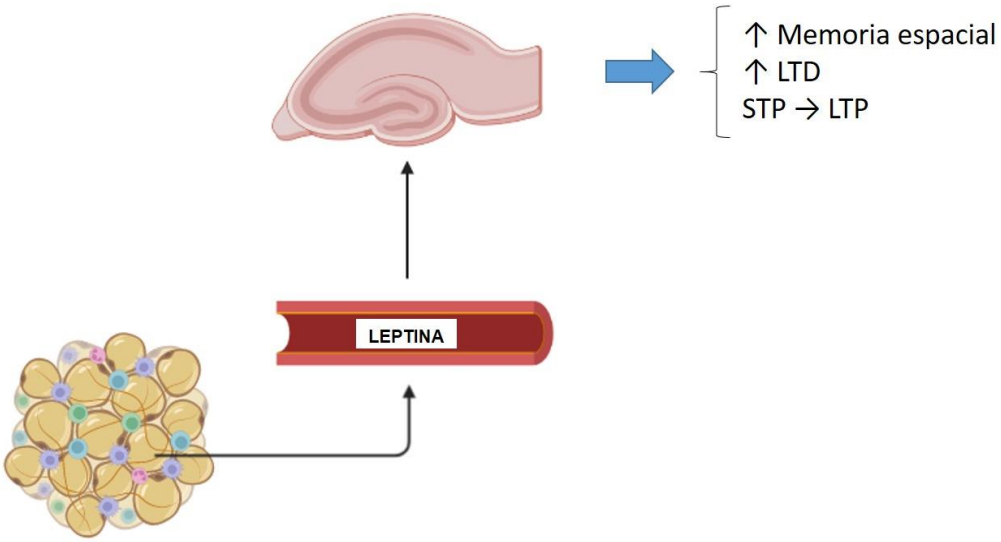


Figura 12. Efectos de la leptina sobre el hipocampo.

Los efectos de la leptina sobre la plasticidad y la memoria hipocampal parecen estar mediados por la vía de la PI3K/Akt (**Tang, 2008**), cuya activación estimula mecanismos similares a la LTD dependiente de NMDA (**Durakoglugil y cols., 2005; Moult y Harvey, 2009**). Además, estudios de nuestro grupo han encontrado que en un contexto de resistencia a leptina hipocampal en la vía de Akt y no en STAT, sólo está comprometida la LTD y no la LTP (**Valladolid-Acebes et al., 2012**), aunque la inhibición farmacológica de STAT3 también puede bloquear la inducción de la LTD (**Nicolas y cols., 2012**). Así, se considera que la integridad de estas vías es necesaria para la regulación por leptina de la plasticidad sináptica. Por otra parte, parece que tiene particular relevancia el receptor de leptina astrocitario, puesto que su delección conduce a una LTD dependiente de NMDA comprometida y una menor homeostasis en el glutamato (**Naranjo y cols., 2019**), tal como se ha encontrado en otras áreas cerebrales como el hipotálamo o la corteza prefrontal (**Hsuchou y cols., 2009; Wang y cols., 2015**). Así, parece que la correcta respuesta a leptina por los astrocitos es esencial para el correcto funcionamiento del hipocampo.

5.4 Hiperleptinemia y resistencia a leptina

El papel de la leptina en la regulación de la ingesta y la homeostasis energética ha atraído mucho interés hacia su posible uso clínico en el tratamiento de la obesidad. Sin embargo, la mayoría de las personas obesas presentan niveles elevados de leptina circulante, mostrando que en el contexto de obesidad se desarrolla resistencia a leptina que conduce al consumo elevado de calorías y previene de la pérdida de peso (**Considine y cols., 1996; Woods y cols., 2000**). Así, muchos de las alteraciones que aparecen tanto en el TAB como en el SNC en el contexto de la obesidad podrían tener un origen endocrino, puesto que la leptina tiene efectos directos sobre ellos y, además, se ha descrito resistencia en todos ellos (**Izquierdo y cols., 2019; Wang y cols., 2000**). Sin embargo los mecanismos responsables de la resistencia aún no están claros; encontrándose además de que según el tipo de dieta se puede desarrollar la resistencia en ausencia de obesidad o hiperleptinemia (**Vasselli y cols., 2013**). De esta manera surge la pregunta de si los efectos derivados del consumo de HFDs están relacionados con la hiperleptinemia, son producto de una acción directa de los ácidos grasos presentes en la dieta o de una combinación de ambos factores.

HIPÓTESIS Y OBJETIVOS

Nuestra **HIPOTESIS** es que el consumo de HFDs tiene un impacto negativo sobre el hipocampo y el hipotálamo, que depende i) de la composición de la dieta y ii) de las alteraciones endocrinas del TAB y, en particular, de la producción excesiva de leptina.

Nuestro principal **OBJETIVO** ha **sido identificar en ratones la influencia del consumo de grasas saturadas y monoinsaturadas sobre el eje tejido adiposo-sistema nervioso central en relación con el desarrollo de resistencia a leptina en el tejido adiposo, el hipocampo y el hipotálamo**.

Los objetivos específicos del estudio, reflejados en las publicaciones adjuntadas, han sido:

I. Caracterizar el efecto de las grasas saturadas y monoinsaturadas sobre el hipocampo, estudiando:
   - **Los mecanismos de plasticidad sináptica relacionados con el aprendizaje y la memoria**.
   - **Los niveles de los aminoácidos implicados en la neurotransmisión**.
   - **La funcionalidad de los receptores de leptina.**

II. Caracterizar el efecto del consumo de grasas saturadas y monoinsaturadas sobre el hipotálamo, estudiando:
   - **La funcionalidad de los receptores de leptina, así como los mecanismos implicados en la regulación de la ingesta.**

III. Caracterizar el efecto del consumo de grasas saturadas y monoinsaturadas sobre el TAB, estudiando:
   - **La expansibilidad y composición del TA**
   - **El efecto autocrino de la leptina.**

PUBLICACIÓN 1: 'SATURATED AND UNSATURATED FAT DIETS IMPAIR HIPPOCAMPAL GLUTAMATERGIC TRANSMISSION IN ADOLESCENT MICE'

Fernández-Felipe J, Merino B, Sanz-Martos AB, Plaza A, Contreras A, Naranjo V, Morales L, Chowen JA, Cano V, Ruiz-Gayo M, Del Olmo N. Saturated and unsaturated fat diets impair hippocampal glutamatergic transmission in adolescent mice. Psychoneuroendocrinology. 2021 Nov;133:105429. doi: 10.1016/j.psyneuen.2021.105429

https://doi.org/10.1016/j.psyneuen.2021.105429

RESUMEN

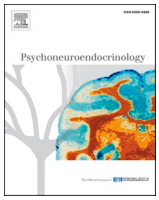
El consumo de dietas enriquecidas en grasas está asociado al deterioro de la plasticidad sináptica y a la aparición de desórdenes cognitivos, todo ello ligado a una alteración del sistema glutamatérgico en el hipocampo. Debido a que los individuos jóvenes son especialmente vulnerables al efecto de nutrientes y xenobióticos sobre la cognición, el **objetivo** de este trabajo ha sido estudiar el efecto del consumo crónico de grasas saturadas (SOLF) y monoinsaturadas (UOLF) sobre 1) la memoria espacial, 2) la transmisión y plasticidad sinápticas hipocampal y 3) la expresión de receptores de glutamato y de hormonas en el hipocampo de ratones adolescentes y adultos. Nuestros **resultados** muestran que tanto SOLF como UOLF perjudican la memoria espacial a corto plazo. Además, los mecanismos de la plasticidad sináptica hipocampal que subyacen a los procesos de memoria, junto a la expresión de los receptores de glutamato, están modulados por ambas dietas. Por otra parte, la expresión génica de PPARγ está específicamente reprimida en los ratones adolescentes que ingirieron SOLF y sobreexpresada en los ratones adultos que consumieron UOLF.

Psychoneuroendocrinology 133 (2021) 105429

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen



Saturated and unsaturated fat diets impair hippocampal glutamatergic transmission in adolescent mice

J. Fernández-Felipe [a,1], B. Merino [a,1], A.B. Sanz-Martos [a], A. Plaza [a,b], A. Contreras [a], V. Naranjo [a], L. Morales [a], J.A. Chowen [c], V. Cano [a], M. Ruiz-Gayo [a], N. Del Olmo [a,d,*]

[a] Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad San Pablo-CEU, Madrid, Spain
[b] Laboratory of Bioactive Products and Metabolic Syndrome (BIOPROMET), IMDEA Food Institute, Madrid, Spain
[c] Departamento de Endocrinología, Hospital Infantil Universitario Niño Jesús, Instituto de Investigación La Princesa, Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Instituto Madrileño de Estudios Avanzados Food Institute (IMDEA), Madrid, Spain
[d] Departamento de Psicobiología, Facultad de Psicología, UNED, Madrid, Spain

ARTICLE INFO

Keywords:
SOLF
UOLF
LTP
LTD
Synaptic plasticity
Memory

ABSTRACT

Consumption of high-fat diets (HFD) has been associated with neuronal plasticity deficits and cognitive disorders linked to the alteration of glutamatergic disorders in the hippocampus. As young individuals are especially vulnerable to the effects of nutrients and xenobiotics on cognition, we studied the effect of chronic consumption of saturated (SOLF) and unsaturated oil-enriched foods (UOLF) on: i) spatial memory; ii) hippocampal synaptic transmission and plasticity; and iii) gene expression of glutamatergic receptors and hormone receptors in the hippocampus of adolescent and adult mice. Our results show that both SOLF and UOLF impair spatial short-term memory. Accordingly, hippocampal synaptic plasticity mechanisms underlying memory, and gene expression of NMDA receptor subunits are modulated by both diets. On the other hand, PPARγ gene expression is specifically down-regulated in adolescent SOLF individuals and up-regulated in adult UOLF mice.

1. Introduction

Consumption of high-fat diets (HFD) has been associated with both neuronal plasticity deterioration and cognitive deficits linked to the impairment of glutamatergic neurotransmission within the hippocampus (HIP) (Del Olmo and Ruiz-Gayo, 2018). These deficiencies are particularly intense in individuals that start to consume this type of diets during pre-adulthood, as demonstrated by studies comparing the effect of HFD in young and adult mice and showing that HFD worsen both relational and spatial memory capacities, specifically in animals that consume these diets during the juvenile period (Boitard et al., 2012; Del Rio et al., 2016; Kaczmarczyk et al., 2013; Valladolid-Acebes et al., 2013, 2011). Memory decline induced by HFD overlaps with the impairment of synaptic efficacy and blunting of NMDA-induced long-term depression (LTD) within the HIP (Valladolid-Acebes et al., 2012). The deterioration of synaptic plasticity triggered by HFD occurs concomitantly with changes in glutamatergic neurotransmission, as assessed in *ex vivo* neurochemical studies showing that HFD consumption decreases the efficiency of glutamate (GLU) up-take and evokes an adaptive up-regulation of glial GLU transporters (GLT-1 and GLAST) as well as a down-regulation of glutamine synthase within the HIP (Valladolid-Acebes et al., 2012).

The above-mentioned changes are apparently not due to the resulting obesity as they are detected before obesity is established and have been linked to brain insulin resistance (Beilharz et al., 2015; Grillo et al., 2015; Vinuesa et al., 2016). Other studies have reported that deficient leptin receptor (LepR) signalling triggered by HFD intake might also account for HIP function impairment (Cordner and Tamashiro, 2015; Dodds et al., 2011; Hwang et al., 2010; Mainardi et al., 2017; McGregor and Harvey, 2018; Valladolid-Acebes et al., 2012). It is suggested that either specific fatty acids (FAs) or a certain ratio of saturated vs. unsaturated FAs yielded by HFD consumption could account for HIP

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid; AMPAR, AMPA receptor; BST, Basal synaptic transmission; BW, Body weight; FA, Fatty acid; fEPSP, Field excitatory postsynaptic potentials; GLU, Glutamate; HFD, High-fat diet; HIP, Hippocampus; LepR, Leptin receptor; NMDA, N-methyl-D aspartate; NMDAR, NMDA receptor; SOLF, Saturated oil-enriched food; UOLF, Unsaturated oil-enriched food.

* Corresponding author at: Departamento de Psicobiología, Facultad de Psicología, UNED, 28040 Madrid, Spain.
E-mail addresses: jesus.fernandezfelipe@beca.ceu.es (J. Fernández-Felipe), nuriadelolmo@psi.uned.es (N. Del Olmo).

[1] These authors equally contribute to this work.

https://doi.org/10.1016/j.psyneuen.2021.105429
Received 19 June 2020; Received in revised form 10 September 2021; Accepted 20 September 2021
Available online 24 September 2021

dysfunction independently of other factors (Del Olmo and Ruiz-Gayo, 2018). Moreover, although no previous study has demonstrated that saturated FA intake is an independent risk factor for memory and mood decline, population studies carried out in children/adolescents point to a negative correlation between HIP-dependent relational memory and saturated fat intake, independently of metabolic factors (Baym et al., 2014). Furthermore, HIP-dependent memory deficits have been observed in adolescent mice that display HFD-induced obesity, but not systemic insulin resistance (Valladolid-Acebes et al., 2011).

Related to the work from other groups (Spinelli et al., 2017), the present study is based on the hypothesis that regular intake of diets containing elevated amounts of saturated, but not unsaturated FAs is detrimental for HIP mechanisms that underlie learning/memory, particularly in individuals that consume these diets during the adolescence/early juvenile period. To address this issue, diets enriched with either saturated (SOLF, **S**aturated **O**i**L**-enriched **F**ood) or unsaturated fat (UOLF, **U**nsaturated **O**i**L**-enriched **F**ood) were provided for 8 weeks to 5-(adolescent) and 8-weekold (young adult) mice, and their effects on: i) spatial short-term memory; ii) synaptic transmission and plasticity, and iii) gene and protein expression of glutamate, hormone and peroxisome proliferator-activated (PPARs) receptors, were characterized in the HIP.

2. Material and methods

2.1. Diets, animals, and experimental design

The two HFDs used in this study were manufactured in our laboratory as previously described (Plaza et al., 2019) from standard rodent chow (60%; SD, Teklad global 2018, Harlan Laboratories, IN, USA) and 40% of either high-oleic sunflower oil (***U**nsaturated **O**i**L**-enriched **F**ood*, UOLF) or palm kernel oil (***S**aturated **O**i**L**-enriched **F**ood*, SOLF) (see diets' composition in Supplementary Material, Table 1).

All experiments were performed in C57BL/6J male mice (Charles River, France). Briefly, mice were housed under a 12-h light/12-h dark cycle, in a temperature-controlled room (22ºC) with water available ad libitum, in accordance with the European Communities Council Directive (86/609/EEC) for the care and use of laboratory animals (PCD-CEU08–112–16). Animals were weaned at P21 and were kept on standard chow until P35, when they were divided into three groups with similar mean body weight (BW), single housed to properly monitor food intake, and assigned (free access) either to SD (Teklad global 2018, Harlan Laboratories, IN, USA), UOLF or SOLF. After 8 weeks, animals were used for the procedures detailed bellow. Mice were killed by decapitation, blood was collected and preserved, and HIP were dissected and used for electrophysiological studies or frozen for further analyses.

Two studies were conducted to identify the differential effect of SOLF and UOLF on **1)** spatial memory performance (Y-maze test) and HIP gene expression of hormone receptors (insulin, leptin and adiponectin), PPARs, and genes involved in glutamatergic transmission, and **2)** basal synaptic transmission (BST) and synaptic plasticity, as well as on protein levels of GLU receptor subunits within the HIP.

Study 1 was carried out simultaneously in mice that started to consume the assigned diet either on P35 (these animals received the diet during the adolescent period and early adulthood and will be referred as the adolescent cohort from now, n = 7 per group) or on P56 (referred as adult cohort, as they consumed the diet only during adulthood, n = 7 per group). These animals were the same used in a previous report (Plaza et al., 2019). **Study 2** (n = 17 per group) was carried out only in adolescent mice (Fig. 1).

2.2. Plasma biochemistry

Plasma leptin, insulin, gastric inflammatory polypeptide (GIP), resistin, interleukin-6 (IL6), tumour necrosis factor α (TNFα), and

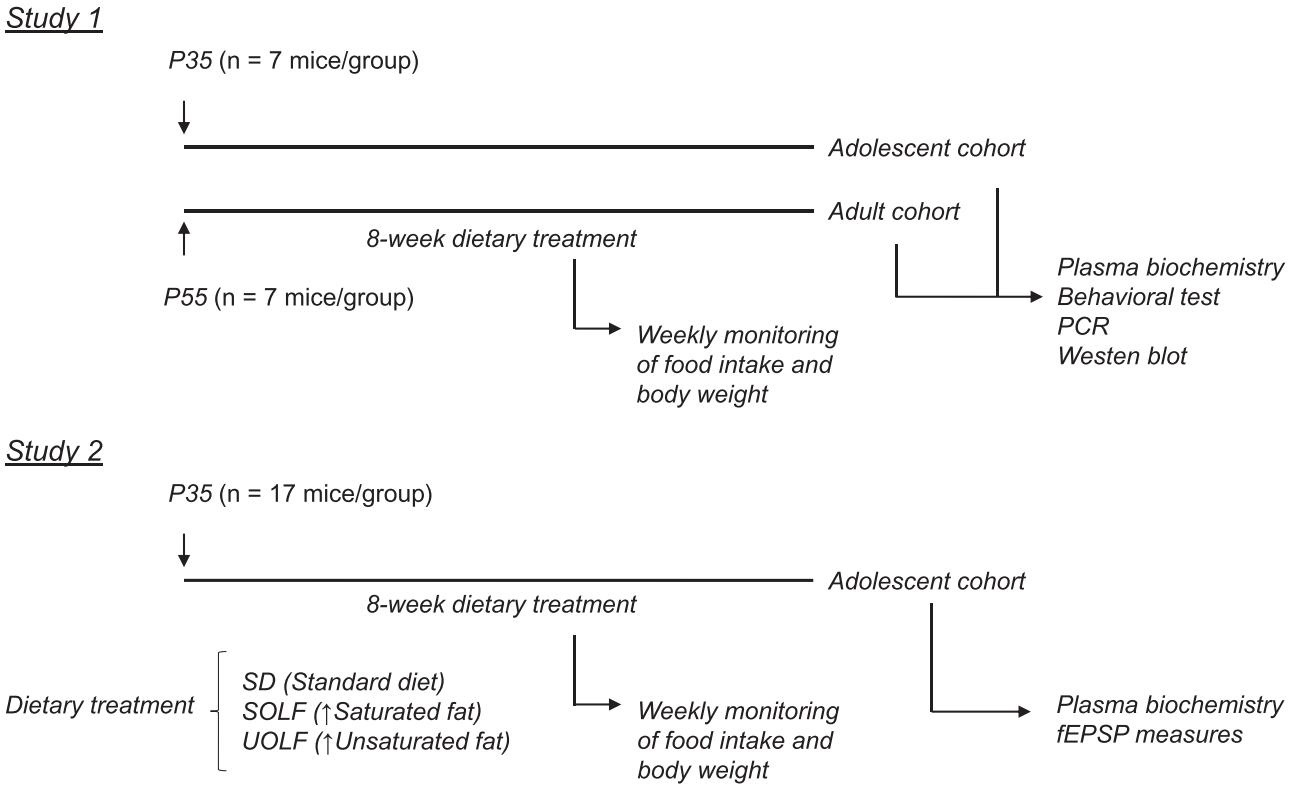


Fig. 1. Schematic illustration of the dietary protocol. The research was organized in two studies. Study 1 was carried out both in adolescent and adult animals that started to consume the experimental diets on postnatal days 35 (P35) and 55 (P55), respectively. Study 2 was carried out only in adolescent animals. In both studies food intake and BW were monitored weekly. Animals from Study 1 were tested in the Y-maze at the end of the dietary treatment, then killed and HIP samples were used for WB and RT-PCR. Animals from Study 2 were used in electrophysiological experiments.

monocyte chemoattractant protein-1 (MCP1) were measured in a multiplex immunoassay (Millipore, Billerica, MA) in a Bio-Plex suspension array system 200 (Bio-Rad Laboratories, Hercules, CA, USA). Adiponectin was analysed by EIA (Abcam, UK). Triglycerides (Spinreact, Spain) and non-esterified FA (NEFA) (Wako Bioproducts, USA) were measured by spectrophotometric methods.

2.3. Evaluation of spatial memory in the Y maze behavioral testing

Spatial short-term working memory was analyzed by recording spontaneous alternation in a black plexiglass Y maze conformed by three identical arms (50 cm long x 19 cm wide) with 35 cm high walls (Contreras et al., 2019). Animals were placed in the testing room, which was rich in visual cues, 60 min before testing for habituation. For testing, mice were placed into one arm, and allowed to explore the maze for 10 min, during which arm entries (all four paws within an arm) and decision time (time in the center of the maze) were recorded. A correct alternation was defined as an entry into three different arms (A, B and C) in overlapping successive sequences of 3 arm entries (e.g., a successive entry sequence A, C, B, C, B, A, C, A, B). The percent alternation score was calculated as [actual alternations]/[possible alternations] X 100.

2.4. Electrophysiology assays

Briefly, transverse HIP slices (400 μm thick) were prepared by using a manual tissue chopper (Stoelting Tissue Slicer, Illinois) and placed in gassed (95% O_2, 5% CO_2) ice-cold Krebs–Ringer bicarbonate (KRB) solution containing (mM); 119 NaCl, 2.5 KCl, 1 KH_2PO_4, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 26.2 $NaHCO_3$ and 11 glucose, in a humidified interface chamber at 20–25ºC, as described previously (Del Olmo et al., 2000, 2003). After 2 h incubation, the slices were transferred to the submersion recording chamber, where they were continuously perfused (2 ml/min) with standard KRB solution. Field excitatory postsynaptic potentials (fEPSPs) were evoked by stimulating Schaffer collateral commissural fibers with biphasic electrical pulses (30–70 μA and 100 μs at 0.033 or 0.066 Hz) delivered through bipolar tungsten insulated microelectrodes (0.5 MΩ) and recorded in the CA1 *stratum radiatum* using tungsten electrodes (1 MΩ). Electrical pulses were generated by a pulse generator Master 8 (AMPI, Israel) and the recording electrode was connected to an AI-402 amplifier (Axon Instruments, USA) connected in turn to a CyberAmp 320 signal conditioner (Axon Instruments, USA). Evoked responses were digitized at 25–50 Hz using a Digidata 1320 A (Axon Instruments, USA) and stored on a Pentium IV IBM-compatible computer using pCLAMP 9.0 software (Axon Instruments, USA). The Schaffer collateral axons contacting the cell population of interest were stimulated every 30 s and after obtaining stable synaptic responses for at least 20 min (baseline period) an experimental protocol was performed. Synaptic plasticity was studied by inducing long-term potentiation (LTP) by four high-frequency stimulation (HFS) trains (100 Hz, 1 s, at test intensity) separated by 20 s. In another set of experiments, slices were perfused with 15 μM NMDA for 6 min to induce NMDA-LTD, following the protocol previously used in our laboratory (Lee et al., 1998; Naranjo et al., 2019; Valladolid-Acebes et al., 2012). Synaptic strength was assessed by measuring the initial slope of the fEPSP and the data were normalized with respect to the mean values of the responses of each animal during the 20 min baseline period. A single slice from each individual animal was considered as n = 1. All electrophysiological experiments were carried out at 31–32 °C. In some of the experiments and during the baseline period, input/output (I/O) curves were applied to evaluate basal synaptic transmission (BST).

2.5. RT-PCR

Total HIP RNA was extracted by using the Tri-Reagent protocol (Sigma, USA). cDNA was then synthesized from 1 μg total mRNA by using a high-capacity cDNA RT kit (BioRad, CA). Quantitative RT-PCR was performed by using designed primer pairs (Integrated DNA Technologies, USA. Table 2, Supplementary Material). SsoAdvanced Universal SYBR Green Supermix (BioRad, CA) was used for amplification according to the manufacturer's protocols, in a CFX96 Real Time System (BioRad). Values were normalized to the housekeeping genes *Actb* and *18 s*. The ΔΔC(T) method was used to determine relative expression levels. Statistics were performed using ΔΔC(T) values (Livak and Schmittgen, 2001).

2.6. Western-blot assays

HIP were homogenized (2 cycles of 50 Hz 1 min each one; TissueLyser, Qiagen, Spain) in 500 μL ice-cold buffer (0.42 M NaCl, 1 mM $Na_4P_2O_7$, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 20% glycerol, 1 mg/ml aprotinin, 1 mg/ml leupeptin, 1 mg/ml N^{α}-tosyl-L-lysine chloromethyl ketone hydrochloride, 20 mM NaF, 1 mM trisodium orthovanadate, and 2 mM phenylmethylsulphonyl fluoride in 20 mM HEPES pH=7.9), submitted to three consecutive freezing/thawing (−80ºC/37ºC) cycles, and centrifuged (10 min, 4ºC). Proteins in the supernatant were quantified (Bradford assay) and their final concentration adjusted to 1 μg/μL in Laemli buffer (0.125 mM Tris, pH=6.8 containing 2% SDS, 25% glycerol, 5% β-mercaptoethanol, and 0.01% blue bromophenol). After boiling (5 min), 15 μL samples were loaded in 6% SDS-polyacrylamide gels and submitted to electrophoresis (glycine 0.2 M and SDS 0.1% in TRIS 0.025 M). Proteins were transferred to 0.2 μm nitrocellulose membranes (Bio-Rad, Spain) by using the Trans-Blot Turbo Transfer System and the corresponding Transfer Pack (Bio-Rad, Spain). Membranes were washed (0.1% non-fat dried milk, 0.5% tween 20 in PBS; 2 ×5 min), blocked (5% non-fat dried milk; 0.5% tween-20 in PBS; 1 h), incubated with primary antibodies/antisera (12 h, 4ºC), and finally re-incubated with appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (30 min, 25 ºC) (Table 3, Supplementary Material). Blots were washed, incubated in chemoluminescence reagents (ECL Prime; GE Healthcare Life Sciences), and the bands detected using the ChemiDoc XRS+ Imaging System (BioRad). To check the uniformity of sample loading, blots were re-incubated with β-actin monoclonal antibody (Affinity Bioreagents, CO).

2.7. Statistical analysis

All data are presented as the means ± S.E.M. Statistical significance was determined by one-way ANOVA (ANOVA-1) followed by Bonferroni's post hoc test or two-way ANOVA (ANOVA-2) when appropriate. Outliers were identified by using the ROUT method (Q = 1%; GraphPad Prism software).

3. Results

3.1. Spontaneous alternation is modulated by diets enriched either in saturated or in unsaturated fatty acids

Spontaneous alternation performance was tested in the Y maze both in adolescent and adult mice that consumed either SOLF or UOLF. The percentage of spontaneous alternation was analyzed in adolescent and adult cohorts after dietary treatment, as illustrated in Fig. 2. Statistical analysis by ANOVA-2 revealed an effect of dietary treatment ($P < 0.05$) while no effect of age nor significant interaction dietary treatment x age were observed.

3.2. SOLF and UOLF regulate the expression of both NMDA2A (Grin2A gene) and NMDA2B (Grin2B gene) subunits of the NMDAR in the HIP

Fig. 3A and B show that dietary treatment had no effect on *Gria1* and *Gria2* gene expression, which suggests that AMPAR subunit levels are not modulated by SOLF/UOLF diets. With respect to the NMDAR subunits (Fig. 3D and E), a down-regulation of both *Grin2A* and *Grin2B* gene

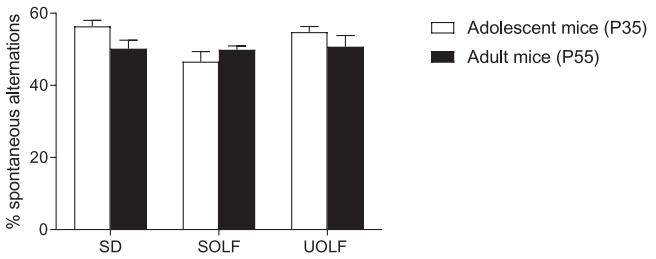


Fig. 2. SOLF impaired memory performance in the Y-maze test specifically in adolescent mice. The graph illustrates the percentage of spontaneous alternations in the Y-maze both in adolescent and adult mice (n = 7 *per* group) after 8-weeks of SOLF and UOLF. Data are expressed as means ± SEM. Comparison was made by ANOVA-2 test (Diet, $F_{(2,38)}$= 3.245, $P < 0.05$; age, $F_{(1,38)}$= 1.617, $P = 0.211$; interaction $F_{(2,38)}$= 2.601, $P = 0.087$).

expression was triggered by the dietary treatment (ANOVA-2; $P < 0.05$ and, $P < 0.05$, respectively). In the case of *Grin2A*, a significant effect of age ($P < 0.01$) was found, whereas no effect in the interaction was detected. For *Grin2B,* both age and the interaction age x diet were not significant (Fig. 3B). No effects on *Grin1* subunit expression were detected (Fig. 3C).

To assess the effect of SOLF on *Grin1, Grin2A and Grin2B* expression, their correspondent encoded proteins were quantified by WB in adolescent mice. As illustrated in Fig. 3G-H, SOLF tended to reduce both NMDA2A and NMDA2B immunoreactivity within the HIP although this effect did not reach statistical significance.

3.3. Peroxisome proliferator activated receptor gamma (Pparg) was down-regulated by SOLF in the hippocampus of adolescent mice

Gene expression of hormone receptors potentially sensitive to HFDs, such as *Ppara* and *Pparg*, insulin (*Insr*), adiponectin (*Adipor1* and *Adipor2*) and leptin (*Lepr*) receptors, was measured in the HIP. In the case of *Ppara,* no effect of the dietary treatment was observed either in adolescent or in adult mice (Fig. 3I). In contrast, *Pparg* expression was dependent on dietary treatment (ANOVA-2; $P < 0.001$), animal age ($P < 0.001$) and interaction *diet x age* $P < 0.001$). *Lepr*, *Adipor* and *Insr* were not affected by the dietary treatment in either adolescent or adult mice (Fig. 3K-N).

3.4. SOLF and UOLF differently affected basal synaptic transmission and LTP/LTD in the hippocampus of adolescent mice

Since previous results, obtained by other authors and ourselves (Boitard et al., 2012; Valladolid-Acebes et al., 2011, 2013), indicate a more potent effect of HFD in adolescent mice, electrophysiological studies were carried out only in this group of animals.

As illustrated in Fig. 4A, both diets affected BST. SOLF blunted input/output (I/O) curves (Fig. 4A) indicating a negative impact of this diet on synaptic efficacy in CA1 pyramidal neurons. A similar effect was triggered by UOLF, although in this case the difference did not reach statistical significance.

The effect of both SOLF and UOLF on HIP synaptic plasticity was further characterized by analyzing their effect on LTP and LTD. Regarding LTP, statistical analysis of the recording by ANOVA-2 indicates significant differences in the factors diet ($P < 0.05$), time ($P < 0.001$) and interaction diet x time ($P < 0.001$; post hoc analysis are included in Fig. 4B). As illustrated in Fig. 4B and C, the magnitude of LTP observed 10 min after HFS was significantly attenuated by SOLF (mean ± S.E.M. of fEPSP 10 min after HFS; SD, 148.9 ± 5.0 vs. SOLF, 113.4 ± 2.5). Moreover, SOLF mitigated LTP maintenance 2 h after induction (mean ± S.E.M. of the fEPSP during the last ten minutes of the recording; SD, 144.6 ± 3.6 vs. SOLF, 117.6 ± 3.2; Fig. 4B and D). In contrast, UOLF failed to inhibit LTP induction (SD, 148.9 ± 5.0 vs. UOLF, 138.6 ± 3.3; ns. 10 min after HFS, Fig. 4B and C), but fully abolished LTP maintenance (last 10 min of the recording; SD, 144.6 ± 3.6 vs. UOLF, 107.9 ± 2.8; Fig. 4B and D). In summary, our results show that LTP induction is specifically impaired by SOLF while UOLF interferes with LTP maintenance.

In regard to NMDA-induced LTD, two-way repeated measures ANOVA (from the beginning until before HFS application) indicated statistical significance in diet, time and interaction diet x time ($P < 0.01$, $P < 0.01$ and $P < 0.001$ respectively; post hoc analysis are shown in the graph). Fig. 5 shows that this mechanism was impaired by UOLF since long-lasting depression of the fEPSP triggered by NMDA was not observed in UOLF-treated mice (SD: 53.2 ± 3.5 vs. UOLF: 85.7 ± 3.3). In contrast, SOLF mice displayed a stronger NMDA-LTD than controls (SD, 53.2 ± 3.5 vs. SOLF, 37.3 ± 2.9).

To further identify the extent to which NMDA responses were due either to synaptic plasticity or to an eventual toxic effect, HFS was applied 50 min after NMDA, once LTD was established (Fig. 5A). In control slices, HFS induced a re-potentiation of fEPSP until baseline values (109.0 ± 5.4, Fig. 5C), which was also observed in UOLF slices (99.8 ± 3.7, Fig. 5C), thus suggesting that HFS was able to produce synaptic plasticity. In contrast, HFS applied to SOLF slices did not evoke any change (65.9 ± 1.5) compared to the Control and UOLF groups; Fig. 5C.) This result would indicate that SOLF-treated HIP present less plasticity, which could be due, at least in part, to the apparent toxic effect of the combination of lauric and palmitic acids contained in SOLF (see LDH cytotoxic assay; Fig. 2, Supplementary Material).

3.5. Plasma biochemistry

Biochemical data from Study 1 have been previously reported (Plaza et al., 2019) and Table 1 shows data corresponding to Study 2. An increase in NEFA values was observed only in SOLF-fed mice ($P < 0.01$), whereas the increase in leptin and insulin was statistically significant only in UOLF mice ($P < 0.05$ and $P < 0.001$, respectively). No changes in GIP, resistin, IL6, TNFα and MCP1 (data not shown), triglycerides and adiponectin were found. BW gain and food intake are shown in Supplementary Material (Fig. 1).

4. Discussion

Our study shows that SOLF and UOLF intake impaired short-term spatial HIP-dependent memory. Moreover, SOLF was more detrimental than UOLF for synaptic plasticity mechanisms underlying spatial memory, such as LTP and LTD. These differences were not dependent on the increase in BW as SOLF caused smaller (this study) or, at most, similar weight gain than UOLF (Plaza et al., 2019). This finding supports the hypothesis that the HIP is a target for saturated FAs. In addition, the vulnerability toward saturated fat was only perceptible in animals that consumed the diet during the juvenile period. To our knowledge, this is the first report studying differential effects of saturated vs unsaturated fat diets on HIP synaptic plasticity mechanisms. It must be highlighted that alternation in the Y-maze is used to interrogate short-term working spatial memory, but additional studies using the radial arm maze or the Morris water maze would be necessary to properly characterize the impairment of learning and memory in response to SOLF/UOLF (Ernyey et al., 2019; Tarantini et al., 2019; Vorhees and Williams, 2014).

We have previously reported that obesity induced by conventional HFDs, which contain elevated amounts of both saturated and unsaturated fat and simple carbohydrates, occurs concomitantly with the impairment of spatial learning and memory in the eight-arm radial maze (Valladolid-Acebes et al., 2011) as well as in the novel recognition test (Valladolid-Acebes, 2013), specifically in mice consuming the diet during the adolescent period. These results, corroborated by other authors also reporting the inhibition of HIP neurogenesis in adolescent mice exposed to HFD (Boitard et al., 2012), are coherent with population studies showing that poor dietary quality is associated with a

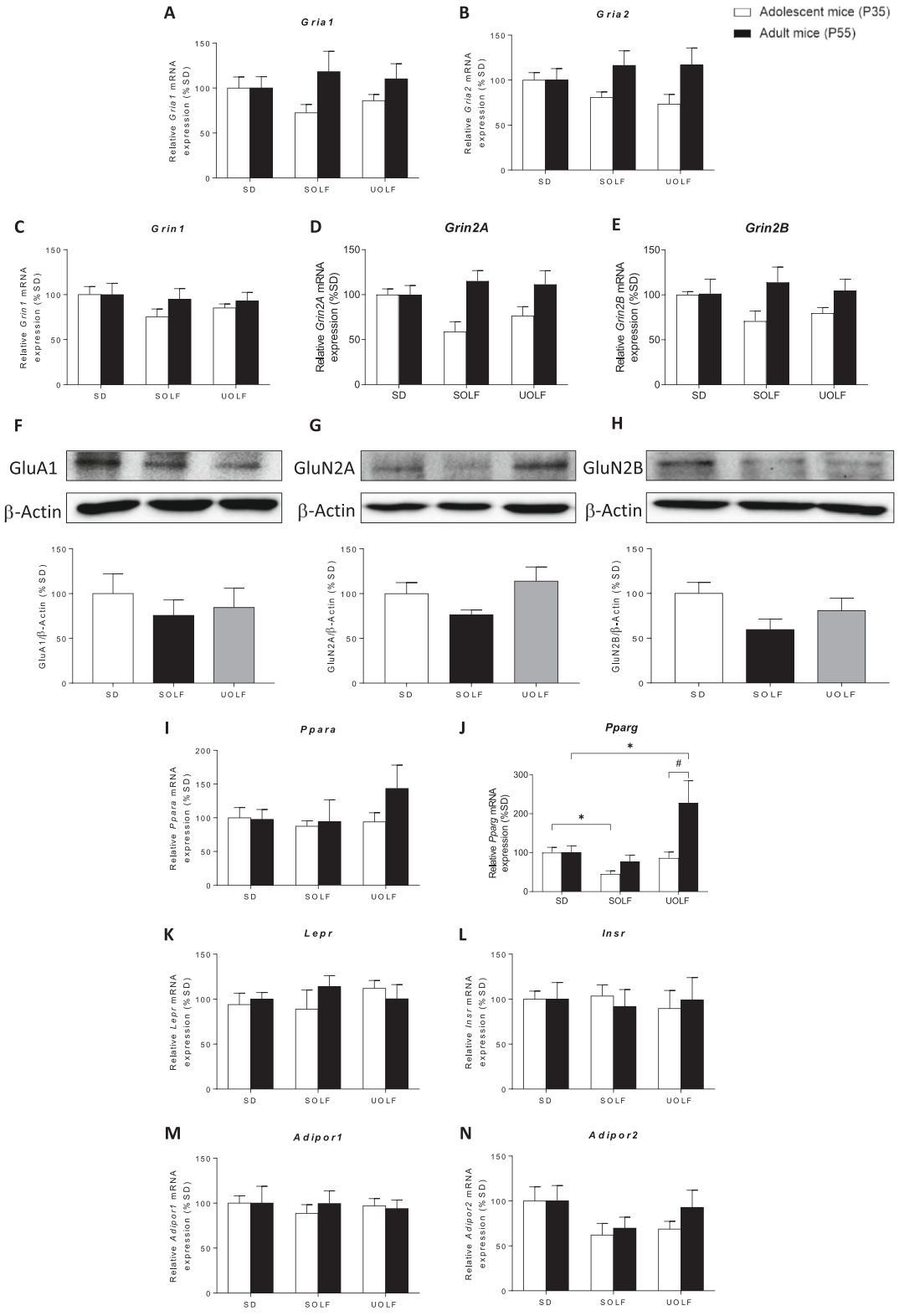


Fig. 3. Effect of 8-week SOLF and UOLF on mRNA levels of glutamate receptors, PPAR, and hormone receptors in adolescent and adult mice hippocampus. Panels A-E illustrate the effect of UOLF and SOLF on gene expression of GLU receptor subunits (*Grin2a*: Diet, $F_{(2,34)}= 2.988$, $P < 0.05$; age, $F_{(1,34)}= 3.113$, $P < 0.01$; interaction, $F_{(2,34)}= 2.163$, $P = 0.063$; *Grin2b*: Diet, $F_{(2,30)}= 2.998$, $P < 0.05$; age $F_{(1,30)}= 1.591$, $P = 0.219$; interaction, $F_{(2,30)}= 1.521$, $P = 0.221$). mRNA levels corresponding to PPARα, PPARγ, leptin, insulin and adiponectin receptors appear illustrated in panels I, J, K, L, M and N, respectively (*Pparg*: Diet, $F_{(2,28)}= 9.552$, $P < 0.001$; age, $F_{(1,28)}= 10.522$, $P < 0.001$; interaction, $F_{(2,28)}= 5.712$, $P < 0.001$, followed by Bonferroni post hoc test; * $P < 0.05$ adolescent SD vs. adolescent SOLF and adult SD vs. adult UOLF; $^{\#}P < 0.05$ adolescent UOLF vs. adult UOLF). Values are means ± SEM (adolescent mice, n = 6 for SD, n = 7 for UOLF and SOLF groups; adult mice, n = 6 for SD, n = 7 for UOLF and SOLF groups). Effect of SOLF/UOLF on GluA1, GluN2A and GluN2B receptor subunit protein levels are shown in panels 3 F, 3 G and 3 H, respectively.

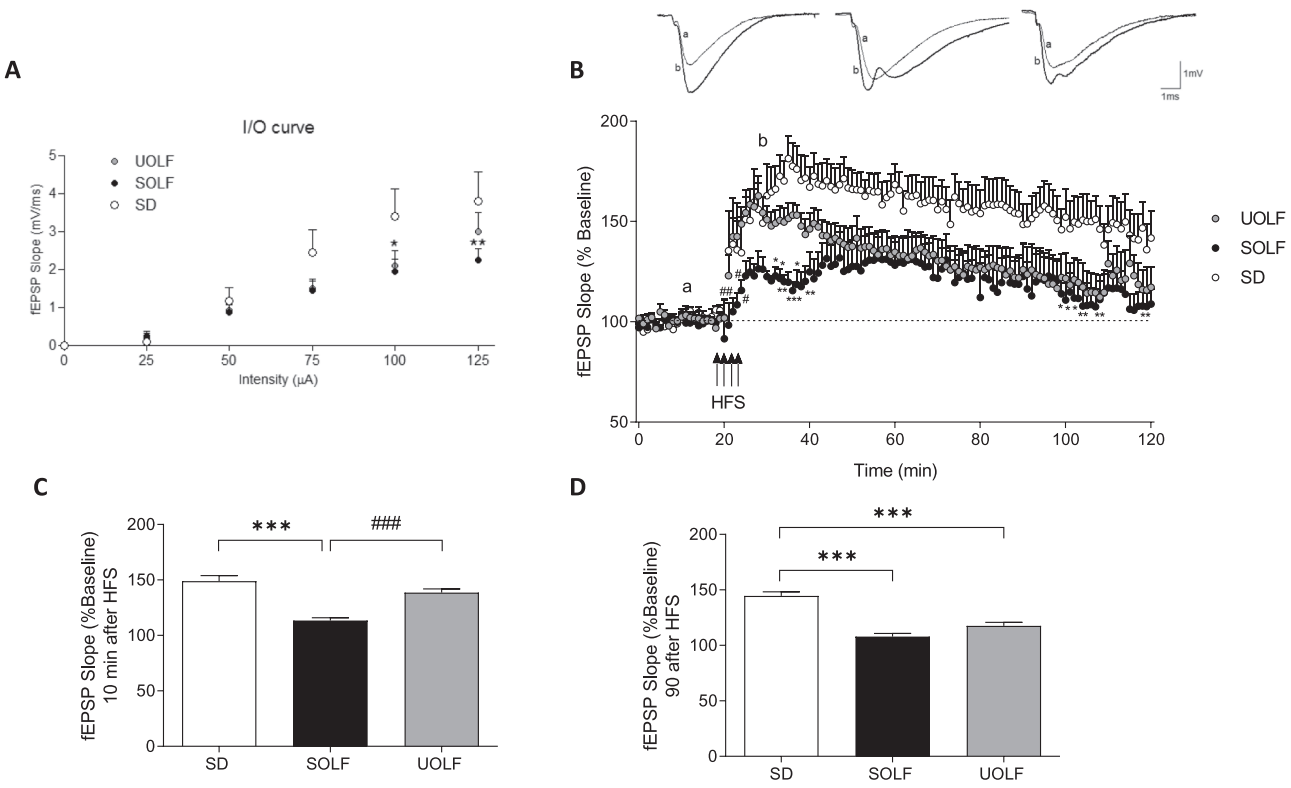


Fig. 4. Synaptic efficacy and long-term potentiation in hippocampus slices after 8-week SOLF and UOLF treatment. (A) Graph plots show stimulus-response curves (means ± S.E.M. of fEPSP slopes vs. stimuli intensity) to measure baseline synaptic responsiveness in HIP slices from SD (white circles, n = 8), SOLF (black circles, n = 10) and UOLF (grey circles, n = 12) treated animals. (Repeated measures ANOVA-2 diet, $F_{(2,30)} = 3.107$, $P < 0.05$; intensity, $F_{(1115,45.45)} = 74.81$, $P < 0.001$; interaction, $F_{(4120)} = 2.994$, $P < 0.01$, followed by Bonferroni post hoc test, $^{*}P < 0.05$ and $^{**}P < 0.01$ SD vs. SOLF). (B) After 20 min of baseline recording, four trains of HFS (indicated by four arrows) were applied to slices from both SD (white circles, n = 9), SOLF (black circles, n = 8) and UOLF-treated animals (grey circles, n = 10). Comparison was made by repeated measures ANOVA-2 (Diet, $F_{(2,23)} = 3.648$, $P < 0.05$; time, $F_{(109,2507)} = 13.93$, $P < 0.001$; interaction, $F_{(218,2507)} = 2.123$, $P < 0.001$ followed by Bonferroni post hoc test * ** $P < 0.001$, * * $P < 0.01$ and * $P < 0.05$ SD vs. SOLF and $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ SOLF vs. UOLF). C and D show the bars representing the mean ± S.E.M. of fEPSP measured for 5 min after 10 min and 90 min of HFS. (ANOVA-1 $F_{(2172)} = 33.05$, $P < 0.001$ and $F_{(2132)} = 67.79$, $P < 0.001$, respectively, followed by Bonferroni post hoc test; $^{***}P < 0.001$ SD vs. SOLF and SD vs. UOLF; $^{\#\#\#}P < 0.001$ SOLF vs. UOLF). Upper traces show representative recordings from one of each type of experiment (a and b show the time points corresponding to each curve). No more than 1 slice/mouse was used in each experiment.

decline of psychological functioning in adolescents (Jacka et al., 2011), and poor learning and memory capacities (Wright et al., 2017). However, none of these studies has identified the contribution to memory impairment of obesity itself vs that of particular nutrients (fat vs sugar) contained in HFD. The current research focusses on the effect of two sucrose/glucose-free diets containing an elevated proportion of either saturated (SOLF) or unsaturated FAs (UOLF); it has to be highlighted that SOLF contains a high proportion of palmitic and, especially, of lauric acid, which adds a supplementary interest to our work, since coconut oil, whose main FA is lauric acid, has been shown to impair memory function and alter HIP morphology in rats (Granholm et al., 2008). Taken together with other studies showing that palmitic acid induces morphological changes in primary HIP cultures, able to compromise cell function and excitability (Loehfelm et al., 2020), and to trigger microglia activation (Tu et al., 2019; Lin et al., 2020), our current data suggest that the combination of lauric and palmitic acids contained in SOLF has a critical role in cognitive deficits associated to SOLF consumption. Moreover, our data corroborate that consumption of HFDs is detrimental and further support the negative impact that regular intake of fat-enriched meals has on cognitive processes. In this sense, other authors have recently shown that acute exposure (7–9 days) to HFD during juvenility is sufficient to impair HIP functions depending on glucocorticoid receptors (Khazen et al., 2019).

The possibility that diet composition might be an obesity-independent triggering factor for cognitive impairment is stressed by other studies concluding that western-style diets cause neurocognitive damage in absence of obesity/overweight (Beilharz et al., 2015). It has to be highlighted that, under our experimental conditions, SOLF mice displayed elevated HOMA indexes (Plaza et al., 2019), a circumstance that might have an impact on memory (Rom et al., 2019). In fact, memory/learning deficits have been shown to be linked to brain insulin resistance (Grillo et al., 2015), a condition that can prematurely develops during HFD interventions before obesity is established (Vinuesa et al., 2016). It has to be noted that, in our study, plasma insulin levels were higher in UOLF than in SOLF mice, a data that needs to be interpreted cautiously considering that mice were not fasted before blood sampling. Therefore, a certain degree of insulin resistance, associated to the intake of saturated fat, might contribute to the effects observed here. In any case, the influence of HIP insulin sensitivity on spatial memory is a controversial matter since HFD has a different impact on HIP insulin sensitivity in males and females, which is not accompanied by intersex differences in terms of spatial memory and HIP excitability (Underwood and Thompson, 2016). We want to emphasize, that, in the current study, we have decided to use only males to minimize the number of animals, but new research aimed at characterizing sex differences concerning SOLF/UOLF effects in cognition and synaptic plasticity will be necessary to properly characterize this issue.

Another circumstance that could account for cognitive deficits is the development of HIP leptin resistance triggered by SOLF. This is a relevant issue considering that leptin has been shown to promote HIP-dependent learning and memory (Van Doorn et al., 2017) and to regulate HIP synaptic transmission (Moult et al., 2010; Moult and Harvey,

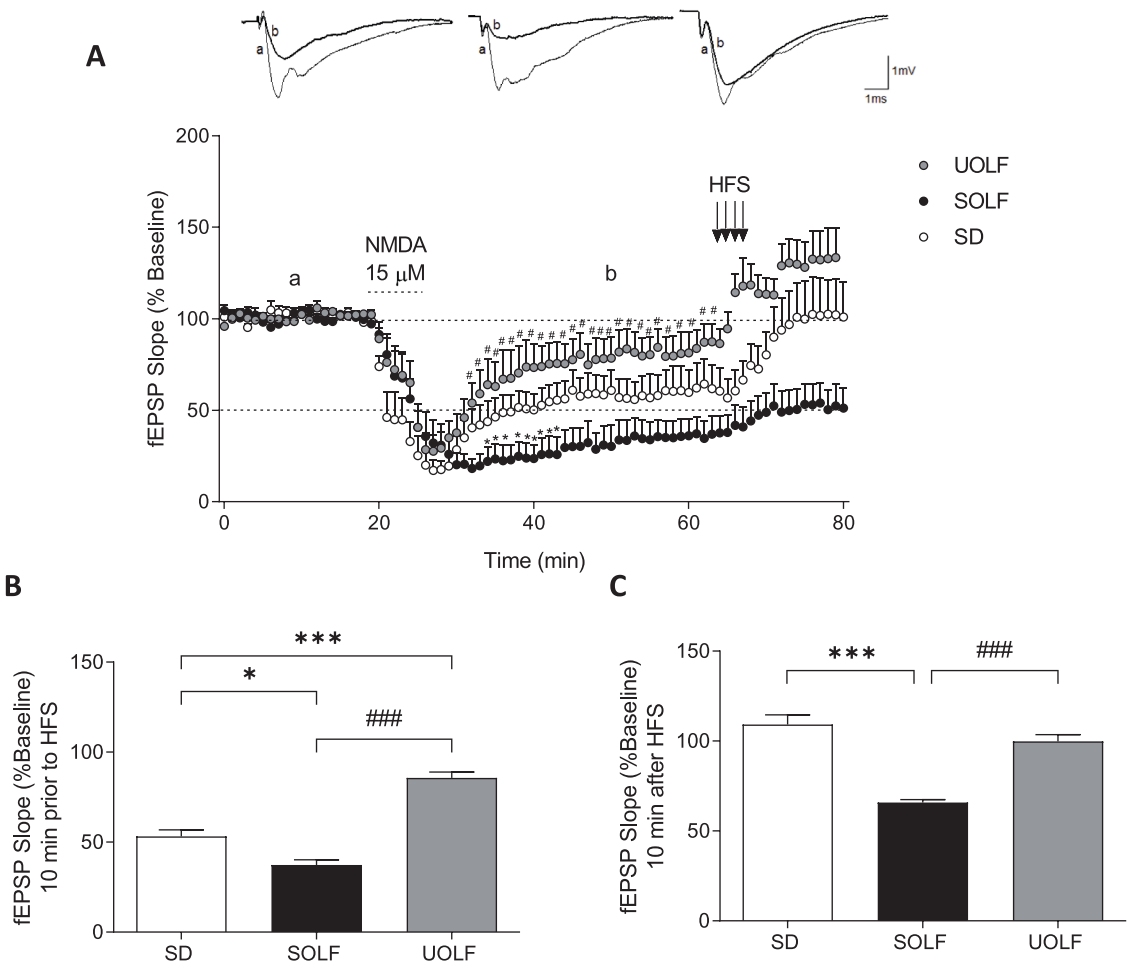


Fig. 5. Long-term depression in hippocampal slices of 8-week SOLF and UOLF treated animals. (A) After 20 min of baseline recordings, 15 μM NMDA was applied (horizontal black line). Forty-five min after NMDA withdrawal, four trains of HFS were applied to slices from SD (white circles, n = 6), SOLF (black circles, n = 8) and UOLF (grey circles, n = 8) animals. (Repeated measures ANOVA-2 was performed until before HFS application (Diet, $F_{(2,16)}$= 7.969, $P < 0.01$; time, $F_{(59,944)}$= 1.771, $P < 0.001$; interaction, $F_{(118,944)}$= 2.423, $P < 0.001$, followed by Bonferroni post hoc test * * $P < 0.01$ and * $P < 0.05$ SD vs. SOLF and $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ SOLF vs. UOLF). Bars represent means ± S.E.M. of fEPSP slopes 10 min prior (B) and 10 min after HFS (C). ANOVA-1 $F_{(2128)}$= 42.51, $P < 0.001$ and $F_{(2140)}$= 34.62, $P < 0.001$, respectively, followed by Bonferroni post hoc test; * ** $P < 0.001$ and * $P < 0.05$ SD vs. SOLF and SD vs. UOLF; $^{\#\#\#}P < 0.001$ SOLF vs. UOLF. Upper traces show representative recordings from one of each type of experiment (a and b show the time points corresponding to each curve). No more than 1 slice/mouse was used in each experiment.

Table 1
Plasma biochemistry.

	SD	SOLF	UOLF
Triglycerides [mg dL^{-1}]	114 ± 7	103.8 ± 3.6	118.9 ± 6.8
NEFA [mg dL^{-1}]	14.87 ± 0.7	17.95 ± 0.5**	15.33 ± 0.6#
Insulin [ng ml^{-1}]	0.82 ± 0.13	1.41 ± 0.23	2.13 ± 0.25***
Leptin [ng ml^{-1}]	1.81 ± 0.34	2.63 ± 0.38	3.66 ± 0.59*
Adiponectin [μg ml^{-1}]	7.85 ± 0.56	6.34 ± 0.73	6.8 ± 0.48

Values are means ± S.E.M. (n = 15/group, except leptin n = 13). Analysis was performed with ANOVA-1 (Triglycerides, F(2,48)= 1.674, P = 0.193; NEFA, F(2,45)= 6.769; P < 0.01; insulin, F(2,34)= 8.416; P < 0.001; leptin, F(2,34)= 4.135; P < 0.05 and adiponectin, F(2,46)= 1.189, P = 0313, all of them followed by Bonferroni post hoc test.

*** P < 0.001.

** P < 0.01.

* P < 0.05 SD vs. SOLF and SD vs. UOLF.

# P < 0.05 SOLF vs. UOLF).

2012; Oomura et al., 2006; Shanley et al., 2001). In fact, leptin-insensitive mice have been shown to display a significant impairment of both LTP and LTD (Li et al., 2002; Winocur et al., 2005), and a desensitization of the protein kinase B (Akt) pathway coupled to HIP leptin receptors (LepR) has been detected in adolescent mice consuming HFD and displaying deficits in spatial memory and moderate hyperleptinemia (Valladolid-Acebes et al., 2013). In the same vein, engineered mice lacking LepR specifically in astrocytes display impaired BST and LTD in the HIP (Naranjo et al., 2019). The possibility that SOLF would promote leptin resistance seems unlikely as plasma leptin levels in adolescent SOLF mice were lower than in their UOLF counterparts, in accordance with previous findings showing that SOLF represses leptin gene expression in visceral WAT (Plaza et al., 2019). However, this possibility cannot be fully discarded inasmuch as diets enriched in saturated fat have been shown to disrupt the blood-brain barrier (Hsu and Kanoski, 2014). Thus, it can be speculated that leptin access to the brain would be increased by SOLF with the consequent development of leptin resistance.

The electrophysiological data suggest that the impairment of spatial memory in SOLF-treated animals is related, at least in part, to the effect of saturated FAs on HIP plasticity and particularly on BST, as previously observed in a study carried out with a classical HFD (Valladolid-Acebes et al., 2012). A surprising result was the shortening of LTP triggered by both SOLF and UOLF, even though SOLF was more potent than UOLF in inhibiting LTP induction. This finding is coherent with studies carried out in animals treated with HFD (Hao et al., 2016), as well as with in

vitro assays showing that HIP slices treated with palmitic acid display a partial inhibition of LTP (Contreras et al., 2017), and confronts with the lack of effect of HFD identified in other studies (Valladolid-Acebes et al., 2012). In any case, the negative impact of UOLF on LTP duration and BST strongly suggests that unsaturated FAs may also have a negative impact on HIP plasticity. Nevertheless, the molecular mechanisms that account for SOLF and UOLF effects in LTP seem to be different, since SOLF affected the induction of the phenomenon whereas UOLF impaired its maintenance. Wong et al. (1989) observed differences between unsaturated and saturated fat-enriched diets in synaptic plasticity mechanisms and observed that a HFD containing both lauric and myristic acids reduced the phosphorylation of the substrate protein F1 (aka GAP43), a factor that accounts for HIP synaptic plasticity (Linden and Routtenberg, 1989). On this basis, one could speculate that a deficient activity of PKC signaling pathways might account for LTP impairment triggered by SOLF.

Regarding LTD, our results are striking considering that it was inhibited by UOLF but apparently potentiated by SOLF. The effect of UOLF, which is similar to that provoked by classical HFDs (Hwang et al., 2010; Valladolid-Acebes et al., 2012), would suggest that unsaturated FAs impair this plasticity mechanism. As changes in NR2A/NR2B ratios have been related to LTD thresholds (Xu et al., 2009), the modulation of NMDAR subunits detected in SOLF-treated mice could underlie changes in synaptic transmission and plasticity. In any case, the effect of SOLF in LTD is difficult to interpret since HFS was unable to re-potentiate the fEPSP slope, as it did in control and UOLF-treated animals. This condition suggests a toxic effect of SOLF, able to exacerbate NMDA-evoked LTD. Such a possibility would be compatible with cytotoxicity detected in HIP slices incubated with a combination of lauric and palmitic acids, used in a proportion similar to that contained in SOLF (Fig. 2, Supplementary Material).

The electrophysiological and behavioural findings could be related to the down-regulation of *Grin2A* and *Grin2B* gene expression triggered by SOLF and UOLF. Such an adaptive regulation is coherent with the specific role of GluN2A and GluN2B in both LTP and LTD (Foster et al., 2010; Kellermayer et al., 2018; Liu et al., 2004; Shipton and Paulsen, 2014). In fact, GluN2A deletion mitigates HIP LTP and impairs spatial learning (Kannangara et al., 2015; Kiyama et al., 1998; Sakimura et al., 1995). In addition, changes in NMDAR density have been detected after synaptic plasticity induction as well as by spatial memory formation (Baez et al., 2018). Some authors point to the increase in the synaptic GluN2A/GluN2B ratio as a stabilizer of synaptic changes, contributing to memory consolidation, particularly concerning spatial representation (Baez et al., 2018). Our data are coherent with this idea and would suggest that modulation of NMDAR subunits by SOLF might underlie the deleterious effect of unsaturated FAs on spatial memory. It must be noted that the inhibition of *Grin2A* and *Grin2B* expression was not accompanied by a significant decrease of the corresponding GluN2A and GluN2B proteins. This circumstance could be due to the fact that protein levels change more slowly than the corresponding mRNA (Vogel and Marcotte, 2013) or even to a poor efficiency of translation mechanism, previously reported by other authors (Boutej et al., 2017; Schindler et al., 1990). In addition, the stability observed in AMPAR subunit mRNA levels (*Gria1* and *Gria2*) also points to the lack of effect of the diets on AMPAR subunit content. The lack of effect of SOLF/UOLF on *Gria1* levels contrasts with the results reported by Spinelli et al. (2017). Although we cannot provide any precise interpretation to explain this difference, one could speculate that SOLF/UOLF and the diet used by Spinelli et al., probably affect GLU turnover (Valladolid-Acebes et al., 2012) differently and trigger a distinct regulation of GLU receptor subunits, as a consequence of specific adaptive compensatory mechanisms. Unfortunately, we lack data regarding the influence of SOLF/UOLF on HIP GLU uptake kinetics that would have allowed proper discussion of this issue.

Finally, the inhibition of PPARγ gene expression suggests that the impairment of synaptic plasticity induced by SOLF could be related to PPARγ modulation, in addition to the decrease in NMDA subunits 2A and 2B. In this regard, our previous studies have shown that commercial HFD containing lard decreases NMDA2B gene expression concomitantly with deficits in spatial learning (Valladolid-Acebes et al., 2011). Moreover, the activation of PPARγ has been demonstrated to ameliorate spatial memory deficits (Chen et al., 2016). For these reasons, further studies using pharmacological tools to investigate whether NMDA and PPARγ agonists prevent/reverse SOLF-evoked spatial memory deficits would be necessary.

Taken together, our data suggest that changes of synaptic plasticity could be mainly related to post-translational modifications of GLU receptors that modulate their functionality; otherwise, one can speculate that changes in plasma levels of insulin and leptin, eventually leading to insulin and/or leptin resistance, might also be pivotal in modulating both HIP synaptic plasticity and short-term memory, particularly in SOLF-treated mice (Harvey, 2007; Harvey et al., 2006). Moreover, PPARγ might also play a role in SOLF effects. In conclusion, our study shows that diets enriched with either saturated or unsaturated FAs modulate spatial HIP-dependent spatial memory through modulation of synaptic transmission and plasticity mechanisms in this area.

Funding

Ministerio de Ciencia e Innovación (BFU2016–78556-R, BFU2017–82565-C21-R2), European Regional Development Fund, Universidad Nacional de Educación a Distancia (UNED) and Fundación Universitaria San Pablo-CEU. A.P. is supported by a grant from Ministerio de Ciencia e Innovación (BFU2016–78556-R). J.F.-F. and A.C. are supported by grants from Fundación Universitaria San Pablo-CEU. A.B. S.-M. is supported by the Youth Employment Initiative (YEI) of Comunidad Autónoma de Madrid.

Acknowledgements

We thank J.M. Garrido, I. Bordallo and J. Bravo for their assistance with animal care.

Disclosure statement

The authors have nothing to disclose.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2021.105429.

References

Baez, M.V., Cercato, M.C., Jerusalinsky, D.A., 2018. NMDA receptor subunits change after synaptic plasticity induction and learning and memory acquisition. Neural Plast. 2018, 5093048 https://doi.org/10.1155/2018/5093048.

Baym, C.L., Khan, N.A., Monti, J.M., Raine, L.B., Drollette, E.S., Moore, R.D., Scudder, M. R., Kramer, A.F., Hillman, C.H., Cohen, N.J., 2014. Dietary lipids are differentially associated with hippocampal-dependent relational memory in prepubescent children. Am. J. Clin. Nutr. 99, 1026–1033. https://doi.org/10.3945/ajcn.113.079624.

Beilharz, J.E., Maniam, J., Morris, M.J., 2015. Diet-induced cognitive deficits: The role of fat and sugar, potential mechanisms and nutritional interventions. Nutrients 7, 6719–6738. https://doi.org/10.3390/nu7085307.

Boitard, C., Etchamendy, N., Sauvant, J., Aubert, A., Tronel, S., Marighetto, A., Layé, S., Ferreira, G., 2012. Juvenile, but not adult exposure to high-fat diet impairs relational memory and hippocampal neurogenesis in mice. Hippocampus 22, 2095–2100. https://doi.org/10.1002/hipo.22032.

Boutej, H., Rahimian, R., Thammisetty, S.S., Béland, L.C., Lalancette-Hébert, M., Kriz, J., 2017. Diverging mRNA and protein networks in activated microglia reveal SRSF3 suppresses translation of highly upregulated innate immune transcripts. Cell Rep. 21, 3220–3233. https://doi.org/10.1016/j.celrep.2017.11.058.

Chen, T., Wang, C., Sha, S., Zhou, L., Chen, Lei, Chen, Ling, 2016. Simvastatin enhances spatial memory and long-term potentiation in hippocampal CA1 via upregulation of α7 nicotinic acetylcholine receptor. Mol. Neurobiol. 53, 4060–4072. https://doi.org/10.1007/s12035-015-9344-6.

Contreras, A., Del Rio, D., Martínez, A., Gil, C., Morales, L., Ruiz-Gayo, M., Del Olmo, N., 2017. Inhibition of hippocampal long-term potentiation by high-fat diets: Is it related to an effect of palmitic acid involving glycogen synthase kinase-3? Neuroreport 28, 354–359. https://doi.org/10.1097/WNR.0000000000000774.

Contreras, A., Polín, E., Miguéns, M., Pérez-García, C., Pérez, V., Ruiz-Gayo, M., Morales, L., Del Olmo, N., 2019. Intermittent-excessive and chronic-moderate ethanol intake during adolescence impair spatial learning, memory and cognitive flexibility in the adulthood. Neuroscience 418, 205–217. https://doi.org/10.1016/j.neuroscience.2019.08.051.

Cordner, Z.A., Tamashiro, K.L.K., 2015. Effects of high-fat diet exposure on learning & memory. Physiol. Behav. 152, 363–371. https://doi.org/10.1016/j.physbeh.2015.06.008.

Dodds, L., Fell, D.B., Shea, S., Armson, B.A., Allen, A.C., Bryson, S., 2011. The role of prenatal, obstetric and neonatal factors in the development of autism. J. Autism Dev. Disord. 41, 891–902. https://doi.org/10.1007/s10803-010-1114-8.

Van Doorn, C., Macht, V.A., Grillo, C.A., Reagan, L.P., 2017. Leptin resistance and hippocampal behavioral deficits. Physiol. Behav. 176, 207–213. https://doi.org/10.1016/j.physbeh.2017.03.002.

Ernyey, A.J., Grohmann Pereira, T., Kozma, K., Kouhnavardi, S., Kassai, F., Gyertyán, I., 2019. Following of aging process in a new motor skill learning model, "pot jumping" in rats. GeroScience 41, 309–319. https://doi.org/10.1007/s11357-019-00073-3.

Foster, K.A., McLaughlin, N., Edbauer, D., Phillips, M., Bolton, A., Constantine-Paton, M., Sheng, M., 2010. Distinct roles of NR2A and NR2B cytoplasmic tails in long-term potentiation. J. Neurosci. 30, 2676–2685. https://doi.org/10.1523/JNEUROSCI.4022-09.2010.

Granholm, A.C., Bimonte-Nelson, H.A., Moore, A.B., Nelson, M.E., Freeman, L.R., Sambamurti, K., 2008. Effects of a saturated fat and high cholesterol diet on memory and hippocampal morphology in the middle-aged rat. J. Alzheimer's Dis. 14, 133–145. https://doi.org/10.3233/JAD-2008-14202.

Grillo, C.A., Piroli, G.G., Lawrence, R.C., Wrighten, S.A., Green, A.J., Wilson, S.P., Sakai, R.R., Kelly, S.J., Wilson, M.A., Mott, D.D., Reagan, L.P., 2015. Hippocampal insulin resistance impairs spatial learning and synaptic plasticity. Diabetes 64, 3927–3936. https://doi.org/10.2337/db15-0596.

Hao, S., Dey, A., Yu, X., Stranahan, A.M., 2016. Dietary obesity reversibly induces synaptic stripping by microglia and impairs hippocampal plasticity. Brain. Behav. Immun. 51, 230–239. https://doi.org/10.1016/j.bbi.2015.08.023.

Harvey, J., 2007. Leptin regulation of neuronal excitability and cognitive function. Curr. Opin. Pharmacol. 7, 643–647. https://doi.org/10.1016/j.coph.2007.10.006.

Harvey, J., Solovyova, N., Irving, A., 2006. Leptin and its role in hippocampal synaptic plasticity. Prog. Lipid Res. 45, 369–378. https://doi.org/10.1016/j.plipres.2006.03.001.

Hsu, T.M., Kanoski, S.E., 2014. Blood-brain barrier disruption: Mechanistic links between western diet consumption and dementia. Front. Aging Neurosci. 6, 1–6. https://doi.org/10.3389/fnagi.2014.00088.

Hwang, L.L., Wang, C.H., Li, T.L., Chang, S.D., Lin, L.C., Chen, C.P., Chen, C.T., Liang, K.C., Ho, I.K., Yang, W.S., Chiou, L.C., 2010. Sex differences in high-fat diet-induced obesity, metabolic alterations and learning, and synaptic plasticity deficits in mice. Obesity 18, 463–469. https://doi.org/10.1038/oby.2009.273.

Jacka, F.N., Kremer, P.J., Berk, M., de Silva-Sanigorski, A.M., Moodie, M., Leslie, E.R., Pasco, J.A., Swinburn, B.A., 2011. A prospective study of diet quality and mental health in adolescents. PLoS One 6, 1–7. https://doi.org/10.1371/journal.pone.0024805.

Kaczmarczyk, M.M., Machaj, A.S., Chiu, G.S., Lawson, M.A., Gainey, S.J., York, J.M., Meling, D.D., Martin, S.A., Kwakwa, K.A., Newman, A.F., Woods, J.A., Kelley, K.W., Wang, Y., Miller, M.J., Freund, G.G., 2013. Methylphenidate prevents high-fat diet (HFD)-induced learning/memory impairment in juvenile mice. Psychoneuroendocrinology 38, 1553–1564. https://doi.org/10.1016/j.psyneuen.2013.01.004.

Kannangara, T.S., Eadie, B.D., Bostrom, C.A., Morch, K., Brocardo, P.S., Christie, B.R., 2015. GluN2A-/- mice lack bidirectional synaptic plasticity in the dentate gyrus and perform poorly on spatial pattern separation tasks. Cereb. Cortex 25, 2102–2113. https://doi.org/10.1093/cercor/bhu017.

Kellermayer, B., Ferreira, J.S., Dupuis, J., Levet, F., Grillo-Bosch, D., Bard, L., Linarès-Loyez, J., Bouchet, D., Choquet, D., Rusakov, D.A., Bon, P., Sibarita, J.B., Cognet, L., Sainlos, M., Carvalho, A.L., Groc, L., 2018. Differential nanoscale topography and functional role of GluN2-NMDA receptor subtypes at glutamatergic synapses. e7 Neuron 100, 106–119. https://doi.org/10.1016/j.neuron.2018.09.012.

Khazen, T., Hatoum, O.A., Ferreira, G., Maroun, M., 2019. Acute exposure to a high-fat diet in juvenile male rats disrupts hippocampal-dependent memory and plasticity through glucocorticoids. Sci. Rep. 9, 1–10. https://doi.org/10.1038/s41598-019-48800-2.

Kiyama, Y., Manabe, T., Sakimura, K., Kawakami, F., Mori, H., Mishina, M., 1998. Increased thresholds for long-term potentiation and contextual learning in mice lacking the NMDA-type glutamate receptor ε1 subunit. J. Neurosci. 18, 6704–6712. https://doi.org/10.1523/jneurosci.18-17-06704.1998.

Lee, W.J., Hawkins, R.A., Viña, J.R., Peterson, D.R., 1998. Glutamine transport by the blood-brain barrier: a possible mechanism for nitrogen removal. Am. J. Physiol. - Cell Physiol. 274, 1101–1107. https://doi.org/10.1152/ajpcell.1998.274.4.c1101.

Linden, D.J., Routtenberg, A., 1989. The role of protein kinase C in long-term potentiation: a testable model. Brain Res. Brain Res. Rev. 14, 279–296. https://doi.org/10.1016/0165-0173(89)90004-0.

Lin, K., Shao, R., Wang, R., Lu, W., Zou, W., Chen, K., Gao, Y., Brietzke, E., McIntyre, R.S., Mansur, R.B., Zhang, L., Yau, S.Y., Su, H., Xu, G., So, K.F., 2020. Inflammation, brain structure and cognition interrelations among individuals with differential risks for bipolar disorder. Brain. Behav. Immun. 83, 192–199. https://doi.org/10.1016/j.bbi.2019.10.010.

Liu, L., Wong, T.P., Pozza, M.F., Lingenhoehl, K., Wang, Y., Sheng, M., Auberson, Y.P., Wang, Y.T., 2004. Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. Science 304, 1021–1024. https://doi.org/10.1126/science.1096615.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Li, X.L., Aou, S., Oomura, Y., Hori, N., Fukunaga, K., Hori, T., 2002. Impairment of long-term potentiation and spatial memory in leptin receptor-deficient rodents. Neuroscience 113, 607–615. https://doi.org/10.1016/S0306-4522(02)00162-8.

Loehfelm, A., Elder, M.K., Boucsein, A., Jones, P.P., Williams, J.M., Tups, A., 2020. Docosahexaenoic acid prevents palmitate-induced insulin-dependent impairments of neuronal health. FASEB J. 34, 4635–4652. https://doi.org/10.1096/fj.201902517R.

Mainardi, M., Spinelli, M., Scala, F., Mattera, A., Fusco, S., D'Ascenzo, M., Grassi, C., 2017. Loss of leptin-induced modulation of hippocampal synaptic trasmission and signal transduction in high-fat diet-fed mice. Front. Cell. Neurosci. 11, 1–11. https://doi.org/10.3389/fncel.2017.00225.

McGregor, G., Harvey, J., 2018. Regulation of hippocampal synaptic function by the metabolic hormone, Leptin: Implications for health and neurodegenerative disease. Front. Cell. Neurosci. 12, 1–11. https://doi.org/10.3389/fncel.2018.00340.

Moult, P.R., Cross, A., Santos, S.D., Carvalho, A.L., Lindsay, Y., Connolly, C.N., Irving, A.J., Leslie, N.R., Harvey, J., 2010. Leptin regulates AMPA receptor trafficking via PTEN inhibition. J. Neurosci. 30, 4088–4101. https://doi.org/10.1523/JNEUROSCI.3614-09.2010.

Moult, P.R, Harvey, J., 2012. Europe PMC Funders Group Europe PMC Funders Author Manuscripts NMDA receptor subunit composition determines the polarity of leptin-induced synaptic plasticity 61, 924–936 doi: 10.1016/j.neuropharm.2011.06.021. NMDA.

Naranjo, V., Contreras, A., Merino, B., Plaza, A., Lorenzo, M.P., García-Cáceres, C., García, A., Chowen, J.A., Ruiz-Gayo, M., Del Olmo, N., Cano, V., 2020. Specific deletion of the astrocyte leptin receptor induces changes in Hippocampus glutamate metabolism, synaptic transmission and plasticity. Neuroscience 447, 182–190. https://doi.org/10.1016/j.neuroscience.2019.10.005.

Del Olmo, N., Galarreta, M., Bustamante, J., Martín Del Río, R., Solís, J.M., 2000. Taurine-induced synaptic potentiation: role of calcium and interaction with LTP. Neuropharmacology 39, 40–54. https://doi.org/10.1016/S0028-3908(99)00078-7.

Del Olmo, N., Handler, A., Alvarez, L., Bustamante, J., Martín Del Río, R., Solís, J.M., 2003. Taurine-induced synaptic potentiation and the late phase of long-term potentiation are related mechanistically. Neuropharmacology 44, 26–39. https://doi.org/10.1016/S0028-3908(02)00310-6.

Del Olmo, N., Ruiz-Gayo, M., 2018. Influence of high-fat diets consumed during the juvenile period on hippocampal morphology and function. Front. Cell. Neurosci. 12, 1–10. https://doi.org/10.3389/fncel.2018.00439.

Oomura, Y., Hori, N., Shiraishi, T., Fukunaga, K., Takeda, H., Tsuji, M., Matsumiya, T., Ishibashi, M., Aou, S., Li, X.L., Kohno, D., Uramura, K., Sougawa, H., Yada, T., Wayner, M.J., Sasaki, K., 2006. Leptin facilitates learning and memory performance and enhances hippocampal CA1 long-term potentiation and CaMK II phosphorylation in rats. Peptides 27, 2738–2749. https://doi.org/10.1016/j.peptides.2006.07.001.

Plaza, A., Antonazzi, M., Blanco-Urgoiti, J., Del Olmo, N., Ruiz-Gayo, M., 2019. Potential role of Leptin in cardiac steatosis induced by highly saturated fat intake during adolescence. Mol. Nutr. Food Res 63, 1900110. https://doi.org/10.1002/mnfr.201900110.

Del Rio, D., Morales, L., Ruiz-Gayo, M., Del Olmo, N., 2016. Effect of high-fat diets on mood and learning performance in adolescent mice. Behav. Brain Res. 311, 167–172. https://doi.org/10.1016/j.bbr.2016.04.052.

Rom, S., Zuluaga-Ramirez, V., Gajghate, S., Seliga, A., Winfield, M., Heldt, N.A., Kolpakov, M.A., Bashkirova, Y.V., Sabri, A.K., Persidsky, Y., 2019. Hyperglycemia-driven neuroinflammation compromises BBB leading to memory loss in both diabetes mellitus (DM) type 1 and type 2 mouse models. Mol. Neurobiol. 56, 1883–1896. https://doi.org/10.1007/s12035-018-1195-5.

Sakimura, K., Kutsuwada, T., Ito, I., Manabe, T., Takayama, C., Kushiya, E., Yagi, T., Shinichi, A., Inoue, Y., Sugiyama, H., Mishina, M., 1995. Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor ε1 subunit. Nature 373, 151–155. https://doi.org/10.1038/373151a0.

Schindler, R., Clark, B.D., Dinarello, C.A., 1990. Dissociation between interleukin-1β mRNA and protein synthesis in human peripheral blood mononuclear cells. J. Biol. Chem. 265, 10232–10237.

Shanley, L.J., Irving, A.J., Harvey, J., 2001. Leptin enhances NMDA receptor function and modulates hippocampal synaptic plasticity. J. Neurosci. 21, 1–6. https://doi.org/10.1523/jneurosci.21-24-j0001.2001.

Shipton, O. a, Paulsen, O., 2014. NMDA receptors in hippocampal plasticity. Philos. Trans. R. Soc. 369, 1–17.

Spinelli, M., Fusco, S., Mainardi, M., Scala, F., Natale, F., Lapenta, R., Mattera, A., Rinaudo, M., Li Puma, D.D., Ripoli, C., Grassi, A., D'Ascenzo, M., Grassi, C., 2017. Brain insulin resistance impairs hippocampal synaptic plasticity and memory by increasing GluA1 palmitoylation through. Nat. Commun. 8, 1–14. https://doi.org/10.1038/s41467-017-02221-9.

Tarantini, S., Yabluchanskiy, A., Csipo, T., Fulop, G., Kiss, T., Balasubramanian, P., DelFavero, J., Ahire, C., Ungvari, A., Nyúl-Tóth, Á., Farkas, E., Benyo, Z., Tóth, A., Csiszar, A., Ungvari, Z., 2019. Treatment with the poly(ADP-ribose) polymerase inhibitor PJ-34 improves cerebromicrovascular endothelial function, neurovascular coupling responses and cognitive performance in aged mice, supporting the NAD+ depletion hypothesis of neurovascular aging. GeroScience 41, 533–542. https://doi.org/10.1007/s11357-019-00101-2.

Tu, T.H., Kim, H., Yang, S., Kim, J.K., Kim, J.G., 2019. Linoleic acid rescues microglia inflammation triggered by saturated fatty acid. Biochem. Biophys. Res. Commun. 513, 201–206. https://doi.org/10.1016/j.bbrc.2019.03.047.

Underwood, E.L., Thompson, L.T., 2016. High-fat diet impairs spatial memory and hippocampal intrinsic excitability and sex-dependently alters circulating insulin and hippocampal insulin sensitivity. Biol. Sex. Differ. 7, 1–15. https://doi.org/10.1186/s13293-016-0060-3.

Valladolid-Acebes, I., Fole, A., Martín, M., Morales, L., Victoria Cano, M., Ruiz-Gayo, M., Olmo, N.Del, 2013. Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin? Neurobiol. Learn. Mem. 106, 18–25. https://doi.org/10.1016/j.nlm.2013.06.012.

Valladolid-Acebes, I., Merino, B., Principato, A., Fole, A., Barbas, C., Lorenzo, M.P., García, A., del Olmo, N., Ruiz-Gayo, M., Cano, V., 2012. High-fat diets induce changes in hippocampal glutamate metabolism and neurotransmission. Am. J. Physiol. - Endocrinol. Metab. 302, 396–402. https://doi.org/10.1152/ajpendo.00343.2011.

Valladolid-Acebes, I., Stucchi, P., Cano, V., Fernández-Alfonso, M.S., Merino, B., Gil-Ortega, M., Fole, A., Morales, L., Ruiz-Gayo, M., Olmo, N.Del, 2011. High-fat diets impair spatial learning in the radial-arm maze in mice. Neurobiol. Learn. Mem. 95, 80–85. https://doi.org/10.1016/j.nlm.2010.11.007.

Vinuesa, A., Pomilio, C., Menafra, M., Bonaventura, M.M., Garay, L., Mercogliano, M.F., Schillaci, R., Lux Lantos, V., Brites, F., Beauquis, J., Saravia, F., 2016. Juvenile exposure to a high fat diet promotes behavioral and limbic alterations in the absence of obesity. Psychoneuroendocrinology 72, 22–33. https://doi.org/10.1016/j.psyneuen.2016.06.004.

Vogel, C., Marcotte, E.M., 2013. Insights into regulation of pr1. Vogel, C. & Marcotte, E. M. Insights into regulation of protein abundance from proteomics and transcriptomis analyses (2013).otein abundance from proteomics and transcriptomis analyses. Nat. Rev. Genet. 13, 227–232 Nat. Rev. Genet. 13, 227–232. https://doi.org/10.1038/nrg3185.Insights.

Vorhees, C.V., Williams, M.T., 2014. Assessing spatial learning and memory in rodents. ILAR J. 55, 310–332. https://doi.org/10.1093/ilar/ilu013.

Winocur, G., Greenwood, C.E., Piroli, G.G., Grillo, C.A., Reznikov, L.R., Reagan, L.P., McEwen, B.S., 2005. Memory impairment in Obese Zucker rats: An investigation of cognitive function in an animal model of insulin resistance and obesity. Behav. Neurosci. 119, 1389–1395. https://doi.org/10.1037/0735-7044.119.5.1389.

Wong, K.L., Murakami, K., Routtenberg, A., 1989. Dietary cis-fatty acids that increase protein F1 phosphorylation enhance spatial memory. Brain Res 505, 302–305. https://doi.org/10.1016/0006-8993(89)91456-x.

Wright, R.S., Waldstein, S.R., Kuczmarski, M.F., Pohlig, R.T., Gerassimakis, C.S., Gaynor, B., Evans, M.K., Zonderman, A.B., 2017. Diet quality and cognitive function in an urban sample: findings from the healthy aging in neighborhoods of diversity across the life span (HANDLS) study. Public Health Nutr. 20, 92–101. https://doi.org/10.1017/S1368980016001361.

Xu, Z., Chen, R.Q., Gu, Q.H., Yan, J.Z., Wang, S.H., Liu, S.Y., Lu, W., 2009. Metaplastic regulation of long-term potentiation/long-term depression threshold by activity-dependent changes of NR2A/NR2B ratio. J. Neurosci. 29, 8764–8773. https://doi.org/10.1523/JNEUROSCI.1014-09.2009.

PUBLICACIÓN 2: 'EFFECTS OF SATURATED VERSUS UNSATURATED FATTY ACIDS ON METABOLISM, GLIOSIS AND HYPOTHALAMIC LEPTIN SENSIVITY IN MALE MICE'

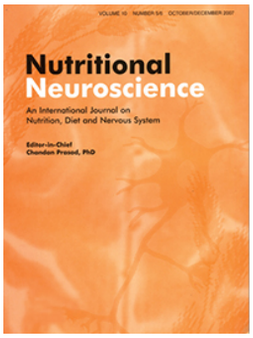
Fernández-Felipe J, Valencia-Avezuela M, Merino B, Somoza B, Cano V, Sanz-Martos AB, Frago LM, Fernández-Alfonso MS, Ruiz-Gayo M, Chowen JA. Effects of saturated versus unsaturated fatty acids on metabolism, gliosis, and hypothalamic leptin sensitivity in male mice. Nutr Neurosci. 2022 Feb 6:1-14.

https://doi.org/10.1080/1028415X.2022.2029294

RESUMEN

El desarrollo de la obesidad y sus comorbilidades no son resultado sólo de un consumo excesivo de energía sino también de la composición de la dieta. De esta manera, cómo interpreta las señales nutricionales los circuitos metabólicos hipotalámicos es esencial para avanzar hacia intervenciones dietéticas efectivas. Por esta razón el **objetivo** de este trabajo ha sido determinar las respuestas metabólicas del hipotálamo frente a dietas enriquecidas en diferentes ácidos grasos. Para ello, ratones macho recibieron una dieta enriquecida en grasas saturadas (SOLF) o monoinsaturadas (UOLF) durante 8 semanas. Tanto SOLF como UOLF aumentaron el peso y la adiposidad de los ratones, no encontrando diferencias entre ambos grupos. Los niveles circulantes de leptina incrementaron por ambas dietas, pero se alcanzaron niveles mayores en los ratones UOLF, tal como ocurre en el tejido adiposo visceral. Al contrario, los niveles de ácidos grasos no esterificados en el suero solamente se incrementaron en los animales SOLF. Los niveles hipotalámicos de ARNm de NPY disminuyeron y los de POMC aumentaron tanto en los ratones SOLF como los UOLF, pero sólo la dieta SOLF mostró señales de astrogliosis y afectación del metabolismo central de ácidos grasos en el hipotálamo. La inyección de leptina exógena activó STAT3 en el hipotálamo de todos los grupos pero la activación de Akt y mTOR y la disminución de la activación de AMPK, que se observó en los animales controles y UOLF, fue ausente en los animales SOLF. Las dietas enriquecidas en ácidos grasos incrementan el peso corporal y la adiposidad incluso sin que la ingesta

energética se incremente, mientras que incremento del consumo de ácidos grasos saturadas y monoinsaturados modifica de forma diferente parámetros metabólicos que podrían subyacer más comorbilidades a largo plazo. De esta manera, es necesario un mayor entendimiento de cómo diferentes nutrientes afectan al metabolismo, ganancia de peso y las complicaciones asociadas a la obesidad.



Taylor & Francis
Taylor & Francis Group

Nutritional Neuroscience

An International Journal on Nutrition, Diet and Nervous System

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ynns20

Effects of saturated versus unsaturated fatty acids on metabolism, gliosis, and hypothalamic leptin sensitivity in male mice

Jesús Fernández-Felipe, Maria Valencia-Avezuela, Beatriz Merino, Beatriz Somoza, Victoria Cano, Ana B. Sanz-Martos, Laura M. Frago, Maria S. Fernández-Alfonso, Mariano Ruiz-Gayo & Julie A. Chowen

To cite this article: Jesús Fernández-Felipe, Maria Valencia-Avezuela, Beatriz Merino, Beatriz Somoza, Victoria Cano, Ana B. Sanz-Martos, Laura M. Frago, Maria S. Fernández-Alfonso, Mariano Ruiz-Gayo & Julie A. Chowen (2022): Effects of saturated versus unsaturated fatty acids on metabolism, gliosis, and hypothalamic leptin sensitivity in male mice, Nutritional Neuroscience, DOI: 10.1080/1028415X.2022.2029294

To link to this article: https://doi.org/10.1080/1028415X.2022.2029294

Published online: 06 Feb 2022.

NUTRITIONAL NEUROSCIENCE
https://doi.org/10.1080/1028415X.2022.2029294

Taylor & Francis
Taylor & Francis Group

Effects of saturated versus unsaturated fatty acids on metabolism, gliosis, and hypothalamic leptin sensitivity in male mice

Jesús Fernández-Felipe[a], Maria Valencia-Avezuela[b], Beatriz Merino[a], Beatriz Somoza[a], Victoria Cano[a], Ana B. Sanz-Martos[a], Laura M. Frago[b,c,d], Maria S. Fernández-Alfonso[e], Mariano Ruiz-Gayo[a] and Julie A. Chowen [b,c,d,f]

[a]Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad San Pablo-CEU, Madrid, Spain; [b]Department of Endocrinology, Hospital Infantil Universitario Niño Jesús, Instituto de Investigación La Princesa, Madrid, Spain; [c]Department of Pediatrics, Universidad Autónoma de Madrid, Madrid, Spain; [d]Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain; [e]Departamento de Farmacología, Farmacognosia y Botánica, Facultad de Farmacia Universidad Complutense de Madrid, Madrid, Spain; [f]IMDEA Food Institute, CEI UAM + CSIC, Madrid, Spain

ABSTRACT

Background: Development of obesity and its comorbidities is not only the result of excess energy intake, but also of dietary composition. Understanding how hypothalamic metabolic circuits interpret nutritional signals is fundamental to advance towards effective dietary interventions.
Objective: We aimed to determine the metabolic response to diets enriched in specific fatty acids.
Methods: Male mice received a diet enriched in unsaturated fatty acids (UOLF) or saturated fatty acids (SOLF) for 8 weeks.
Results: UOLF and SOLF mice gained more weight and adiposity, but with no difference between these two groups. Circulating leptin levels increased on both fatty acid-enriched diet, but were higher in UOLF mice, as were leptin mRNA levels in visceral adipose tissue. In contrast, serum non-esterified fatty acid levels only rose in SOLF mice. Hypothalamic mRNA levels of NPY decreased and of POMC increased in both UOLF and SOLF mice, but only SOLF mice showed signs of hypothalamic astrogliosis and affectation of central fatty acid metabolism. Exogenous leptin activated STAT3 in the hypothalamus of all groups, but the activation of AKT and mTOR and the decrease in AMPK activation in observed in controls and UOLF mice was not found in SOLF mice.
Conclusions: Diets rich in fatty acids increase body weight and adiposity even if energy intake is not increased, while increased intake of saturated and unsaturated fatty acids differentially modify metabolic parameters that could underlie more long-term comorbidities. Thus, more understanding of how specific nutrients affect metabolism, weight gain, and obesity associated complications is necessary.

KEYWORDS
Saturated fatty acids; monounsaturated fatty acids; purified high-fat diets; gliosis; metabolism; leptin resistance

Introduction

The accumulation of adipose tissue results from energy intake exceeding energy expenditure over time and this can eventually result in obesity and its associated complications. Regulation of energy balance (intake versus expenditure) is complex with the outcome being determined by numerous factors including genetics, epigenetics and the prenatal and postnatal environments. Indeed, the propensity to become obese clearly differs between individuals, as does the development of secondary complications associated to this disease such as type 2 diabetes, cardiovascular disease, as well as an increased risk for other conditions such as cancer or neurodegenerative diseases [1, 2]. However, the metabolic compromise and development of associated comorbidities can differ between patients with obesity, even amongst those with the same degree of adiposity [3, 4] and this can be explained in part by the duration of the obese state, as well as genetic components. In addition, it is thought that dietary quality/composition, not just energy intake, can influence not only weight gain but also the development of obesity-associated complications [5, 6].

The chronic inflammatory state associated with obesity has been implicated in the development of its secondary complications [7–9]. For example, infiltration by and activation of macrophages in adipose tissue is associated with insulin resistance in this tissue.

CONTACT Mariano Ruiz-Gayo ruigayo@ceu.es Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad San Pablo-CEU, Madrid 28925, Spain; Julie A. Chowen julieann.chowen@salud.madrid.org, jachowen@gmail.com Department of Endocrinology, Hospital Infantil Universitario Niño Jesús, Avenida Menéndez Pelayo 65, Madrid 28009, Spain

Moreover, hypothalamic inflammation is reported to rapidly occur in response to high fat diet (HFD) intake, even before excess weight gain is detected [10]. This central inflammation is associated with gliosis involving both astrocytes and microglia and is thought to underlie the disarray of systemic glucose metabolism [7, 11–14]. More recently, obesity associated astrogliosis has also been implicated in cardiovascular complications [15, 16]. Glial cell activation differs depending upon the type of fatty acid to which they are exposed [17, 18]; however, the analysis of the differential effects of fatty acids have been performed in vitro, while in vivo studies have largely employed commercial HFD rich in animal fat. Thus, little is known regarding the differential effects of the consumption of specific fatty acids on hypothalamic gliosis/inflammation and hypothalamic metabolic circuits and how this relates to modifications in systemic metabolic parameters.

The commercially produced HFDs that are frequently used for diet-induce weight gain studies are generally composed of saturated fatty acids of animal origin and constituting 45% or even 60% of the energy content. Although useful for the induction of obesity, these diets are not necessarily representative of common dietary habits in the developed world. Considering the current obesity epidemic, the importance of dietary composition in combating this disease, or at least reducing the health-related problems, has received increasing attention and understanding the metabolic consequences of specific nutrients is of utmost importance to improve the overall health of our society. Current dietary recommendations indicate that in adults, lipids should form between 20-35% of the total energy intake, with saturated fats being less than 10% of the dietary intake and with trans-fat intake being as low as possible (https://www.dietaryguidelines.gov/sites/default/files/202103/Dietary_Guidelines_for_Americans-2020-2025). Indeed, saturated fats are accepted as being more dangerous for our health, while we are encouraged to consume 'healthy fats'. However, there is still much to be learned regarding how excess intake of specific fatty acids affects not only weight gain, but our overall health.

Here we have employed diets consisting of normal rodent chow enriched with high-oleic acid sunflower oil or palm kernel oil, fats that are commonly ingested in our diets and that are rich in unsaturated fatty acids or saturated fatty acids, respectively. The effects of eight weeks of these diets on weight gain and metabolic parameters, as well as modifications in the hypothalamic metabolic circuits and inflammatory markers were investigated.

Materials and methods

Animals and experimental protocol

All experiments were carried out in male C57BL/6J mice (Charles River, Saint-Germain-Nuelles, France) that were switched from their normal chow diet to the fatty acid-enriched diets at 5 weeks of age. Mice were housed under 12-h light/12-h dark cycle, in a temperature-controlled room (22°C) with water available *ad libitum*. The Institutional Animal Care and Use Committee approved all experimental procedures according with the European Union Laboratory Animal Care Rules (86/609/EEC) and were approved by the Animal Research Committees of San Pablo CEU University and Complutense University (PCD-CEU08-112-16 and PROEX-017/18, respectively) and the Community of Madrid on 23 March 2017 and 10 April 2018, respectively. All efforts were made to avoid animal suffering in accordance with the ARRIVE guidelines for reporting experiments involving animals [19, 20]. All experimental procedures were performed using coded samples so that the experimental group was unknown to the person performing each analysis.

At the start of each experiment, the mice were divided into three groups with similar mean body weights (BW), single housed, and given free access to the assigned diet for 8 weeks. These diets consisted of either a standard chow diet (SD, 18% energy from fat; Teklad global 2018, Harlan Laboratories, Indianapolis, IL, U.S.A.) or a high fat diet (HFD) enriched in: (1) monounsaturated fat (high oleic sunflower oil -HOSO-, Unsaturated OiL-enriched Food, UOLF, 70% energy from fat) or (3) saturated fat (palm kernel oil, Saturated OiL-enriched Food, SOLF, 70% energy from fat). UOLF and SOLF diets were elaborated by mixing standard chow diet (60%) and 40% of either HOSO or palm kernel oil, respectively, as previously described as well as the nutritional composition [21].

In the first experiment, body weight and food intake were monitored once a week during the initial 7 weeks in mice exposed to chow ($n = 8$), UOLF ($n = 10$), or SOLF ($n = 10$). Caloric intake was calculated employing the energy densities for SD (3.09 kcal/g; 12.94 kjul/g), SOLF (5.3 kcal/g; 22.2 kjul/g) and UOLF (5.3 kcal/g; 22.2 kjul/g)[21]. After 8 weeks of the fatty-acid enriched diets, mice were euthanized, exsanguinated by decapitation and blood, brain, white adipose tissue (WAT) collected.

Assessment of central leptin sensitivity

To determine whether central leptin signaling was affected by dietary intake, a second experiment was

performed where after 8 weeks on either the SD (n = 12), SOLF (n = 12) or UOLF (n = 12), recombinant murine leptin (1 mg/kg) or saline was administered intraperitoneally at 0900 h. We have previously employed this dose, which results in supraphysiological circulating leptin levels, to determine the response to this hormone [22]. After 60 min, mice were killed by decapitation and the hypothalami were dissected and stored at −80°C until assayed. Tissues were prepared for Western blotting as described below.

Plasma biochemistry

Circulating levels of insulin, leptin, IL-6, TNF-α, MCP1 and GIP were determined by a multiplexed magnetic bead immunoassay according to the manufacturer's instructions (Merck Millipore, Burlington, MA, U.S.A.), with all samples being measured in duplicate. Beads conjugated to the appropriate antibodies and serum samples were incubated at room temperature with moderate shaking for 2 h. After washing the wells, the biotinylated detection antibody was added and incubated for 1 h (RT and shaking). The reporter dye, streptavidin-conjugated phycoerythrin, was then added and incubated for 30 min. The beads were washed and analyzed in a Bio-Plex suspension array system 200 (Bio-Rad Laboratories, Hercules, California, U.S.A.). The resulting mean fluorescence intensity was analyzed using Bio-Plex Manager Software 4.1.

Plasma glucose (GTM, Roche, Basel, Switzerland), triglycerides (Spinreact, Vall d'en Bas, Gerona, Spain) and non-esterified free fatty acids (NEFA; Wako Bioproducts, Osaka, Japan) were measured by standard spectrophotometric methods according to the manufacturer's instructions.

Protein and RNA extraction

Total RNA was extracted from each hypothalamus by using RNeasy Plus Mini Kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions and from visceral (VAT) and subcutaneous (SCAT) adipose tissue by using the Tri-Reagent protocol (Sigma, Darmstadt, Germany) followed by purification with RNAspin Mini columns (GE Healthcare, Buckinghamshire, UK). The concentration and purity of RNA were assessed with NanoDrop™ 2000/c (Fisher Scientific, Pittsburgh, PA, U.S.A.).

Protein was isolated from the hypothalamic samples by collecting the first elution from the RNeasy® Mini Spin columns. The eluate was mixed with 4 volumes of cold acetone and stored overnight at −20°C. The samples were then centrifuged at 3,000 rpm at room temperature for 10 min. The acetone was removed, and the pellets resuspended in CHAPS hydrate (7 M urea, 2 M thiourea, 4% CHAPS, 0.5% 1 M Tris pH 8.8). For protein extraction from adipose, the tissue was homogenized in ice-cold buffer containing 0.42 M NaCl, 20 mM HEPES (pH 7.9), 1 mM $Na_4P_2O_7$, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 20% glycerol, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 20 mM sodium fluoride, 1 mM trisodium orthovanadate, and 2 mM phenylmethylsulfonyl fluoride. The homogenates were frozen at −80°C and thawed at 37°C three consecutive times, then centrifuged for 10 min at 4°C and the supernatant removed. Protein concentrations were determined by using Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories) and the samples were stored at −80°C until used.

Real Time qPCR

For RT–PCR in hypothalamic samples, 0.5–1 μg of RNA was retro-transcribed to cDNA by using a NZY First-Strand cDNA Synthesis Kit (NZY Tech, Lisbon, Portugal). For analysis of the hypothalamus, TaqMan probes (Applied Biosystems, Waltham, MA, U.S.A.) including neuropeptide Y (NPY; Mm03048253_m1), Agouti-related protein (AgRP; Mm00475829_g1), proopiomelanocortin (POMC; Mm00435874_m1), leptin receptor (LepR; Mm00440181_m1), carnitine palmitoyltransferase 1A (cpt1a), fatty acid synthase (Fasn), IL6 (Mm00446190_m1), and TNFα (Mm00443260_g1) were used for RT–PCR in a QuantStudio 3 Real-Time PCR System (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous housekeeping control. In adipose tissue, RT-qPCR analysis for leptin expression was performed by synthesizing cDNA from 1 μg total mRNA by using a high-capacity cDNA RT kit (Bio-Rad). Designed primer pairs (Integrated DNA Technologies, Coralville, IA, U.S.A.) and SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) were used for amplification according to the manufacturer's protocols in a CFX96 Real Time System (Bio-Rad). The primer pairs used were as follows: Leptin forward: 5'-GGCTTTGGTCCTATCTGTCTTATGTTC-3' reverse: 5'-CCTGTTGATAGACTGCCAGAGTCTG -3'; 18S forward: 5'-GGGAGCCTGAGAAACGGC-3' reverse: 5'-GGGTCGGGAGTGGGTAATTT-3'; Actb forward: 5'-TGGTGGGAATGGGTCAGAAGGACTC-3' reverse: 5'-CATGGCTGGGGTGTTGAAGGTCTCA-3'. Values were normalized to the housekeeping genes *Actb* and *18s*. All samples were run in duplicate and the ΔΔC(T) method was used to determine relative expression levels. Statistics were performed using ΔΔC

(T) values normalized to control (chow diet) levels in each assay.

Western blotting

Western blotting was performed as previously reported [23]. Briefly, equivalent amounts of proteins present in the supernatant were size-separated by SDS-PAGE. Proteins were transferred to polyvinylidene difluoride membranes (GE Healthcare) using a transblot apparatus (Bio-Rad). For immunoblotting, membranes were blocked with 5% nonfat dried milk in Tween-PBS for 1 h. Primary antibodies (Table 1) were applied at the appropriate dilution overnight at 4° C. After washing, appropriate secondary antibodies were applied for 1 h at a dilution of 1:2,000. Blots were washed, incubated in enhanced chemiluminescence reagent and the resulting bands were detected by using a ChemiDoc XRS+ Imaging System (BioRad) or ImageQuant 4000 (GE Healthcare). Each protein was normalized to GAPDH, vinculin or β-actin levels, in the same sample, while pJNK, pSTAT3, pAKT, pmTOR and pAMPK were also normalized with total levels of JNK, STAT3, AKT, mTOR and AMPK, respectively.

Table 1. Primary antibodies used in Western blotting.

Antigen	Manufacturer	Reference	Host and Molecular weight	Dilution
β-actin	Sigma	#A5316	Mouse mooclonal, 42 KDa	1/5000
CHOP	Santa Cruz Biotechnology	Sc-7351	Mouse monoclonal, 30 kDa	1/1000
Elfα2	Santa Cruz Biotechnology	Sc-11386	Rabbit polyclonal, 36 kDa	1/1000
GAPDH	Sigma	#G9545	Rabbit polyclonal, 37kDa	1/1000
GFAP	Sigma	#G3893	Mouse monoclonal, 50 kDa	1/5000
Iba1	Synaptic System	#234 004	Rabbit, 17 kDa	1/1000
AKT	Cell Signaling	#9272	Rabbit polyclonal, 60	1/500
pAKT (S473)	Cell Signaling	#9271	Rabbit polyclonal, 60 kDa	1/500
AMPK	Cell Signaling	#2532	Rabbit polyclonal, 62 kDa	1/500
pAMPK (T172)	Cell Signaling	#2531	Rabbit polyclonal, 62 kDa	1/500
JNK	Santa Cruz Biotechnology	Sc-1648	Mouse monoclonal, 45,54 kDa	1/1000
pJNK	Promega	#V7932	Rabbit polyclonal, 46,54 kDa	1/3000
STAT3	Santa Cruz Biotechnology	Sc-483	Rabbit polyclonal, 86 kDa	1/250
pSTAT3 (Y705)	Cell Signaling	#9131	Rabbit polyclonal, 86 kDa	1/250
mTOR	Cell Signaling	#4571	Mouse monoclonal, 289 kDa	1/750
pmTOR (S2448)	Cell Signaling	#2971	Rabbit polyclonal, 289 kDa	1/750
Vinculin	Santa Cruz	sc-73614	Mouse monoclonal 117 kDa	1:1000
Anti-mouse	GE Healthcare	NA931V	Sheep whole antibody, HRP	1/1000
Anti-rabbit	Santa Cruz Biotechnology	Sc-2357	Mouse monoclonal, HRP	1/1000

Statistical analysis

All statistics were performed using GraphPad Prism software (GraphPad Software Inc. La Jolla, CA, U.S.A.; Version 7.0a). Normal distribution and variance homogeneity were assessed by means of the Bartlett and Brown–Forsyte test. Time-course of food intake, BW, and caloric efficiency were analyzed by two-way ANOVA with repeated measures with *posthoc* Bonferroni analysis. Individual effects were analyzed by one-way ANOVA followed by Bonferroni post-hoc analysis. Data are expressed as mean ± S.E.M. and statistical significance was set at $p < 0.05$.

Results

Effects of SOLF and UOLF on body weight and energy intake

Throughout the study, the mean grams of food ingested was significantly higher in the control group compared to the groups ingesting a HFD (diet effect; $F_{(2, 27)}$ 182; $p <$ 0.001; Figure 1A). However, there was no difference between groups in energy intake over time (Figure 1B). The cumulative intake of kilocalories and specific fatty acids after 8 weeks of dietary change was calculated from the total grams of food consumed and the composition of these diets as previously reported [21]. Total kilocalorie intake at the end of the study was not different between the SOLF and UOLF groups (Table 2). On a standard diet mice had a higher intake of carbohydrates and protein, with a lower total lipid intake compared to the HFDs, with no difference between these two diets in the total amount of these nutrients. However, the consumption of specific fatty acids was clearly different between the experimental groups, with the intake of oleic acid being significantly higher in the UOLF group compared to the other two experimental groups and with SOLF mice ingesting more oleic acid than controls. Palmitic acid intake was higher in SOLF mice compared to the other two experimental groups and with UOLF mice ingesting more palmitic acid than controls. Levels of lauric acid intake were elevated in the SOLF group, with this fatty acid being undetectable in the other diets.

Although no differences in total energy intake were found, there was an effect of diet on weight gain

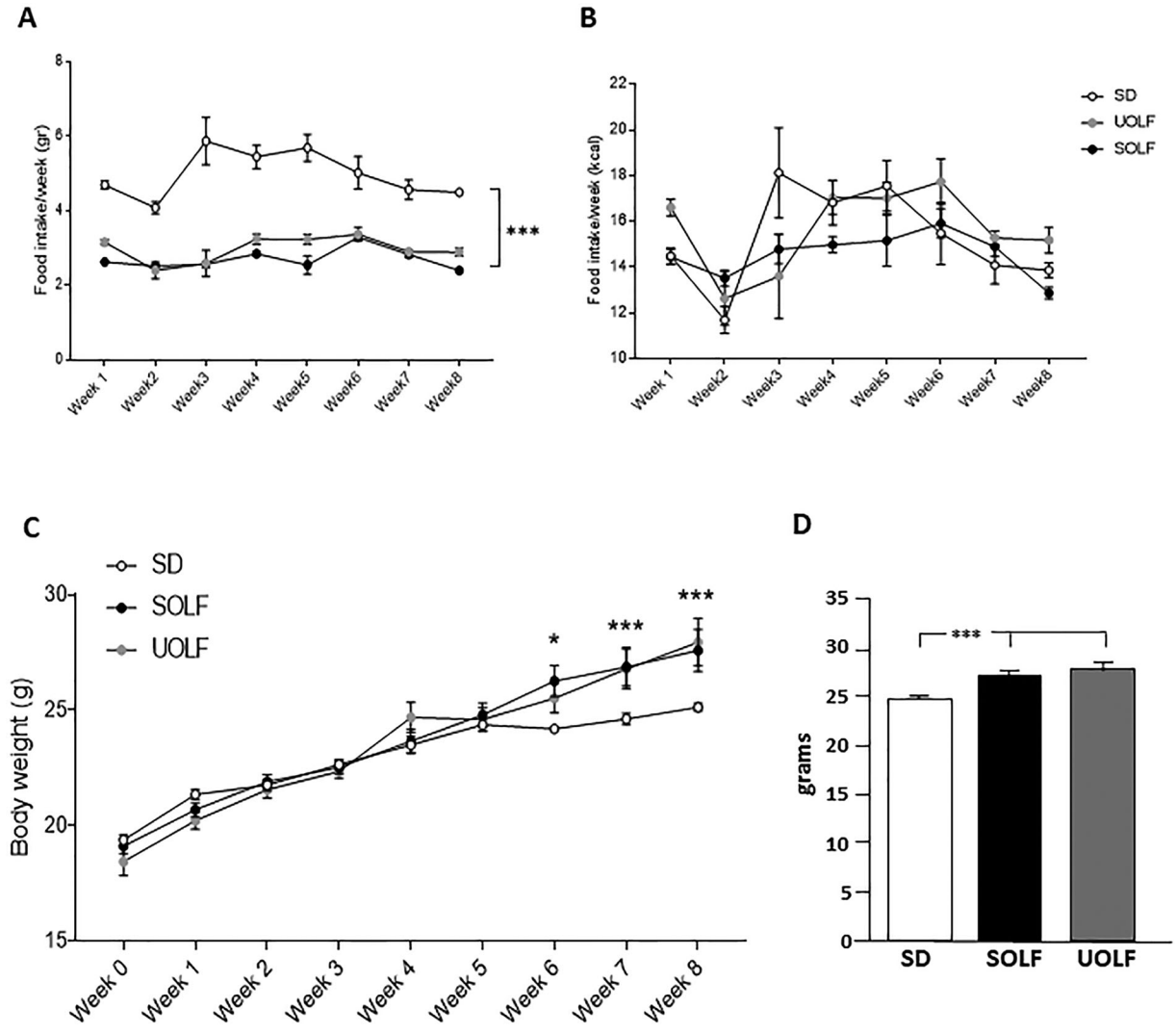


Figure 1. Grams of food (A), energy intake (B) food intake and body weight over time (C) and final body weight (D) in mice on a standard diet (SD) or diets enriched with high oleic sunflower oil, (Unsaturated OiL-enriched Food, UOLF) or palm kernel oil (Saturated OiL-enriched Food, SOLF). Data are shown as mean ± SEM of 8–10 animals per group. *$p < 0.05$, **$p< 0.01$, ***$p< 0.001$ of SOLF and UOLF mice versus the control group (SD).

(Figure 1C; $F_{(2,27)}$ 4.297; $p < 0.01$) with mice that consumed UOLF or SOLF gaining more weight compared to controls. The mean weight of these two experimental groups were significantly higher than the controls from week 5 onward. At the end of the study there was no difference in BW between the groups that consumed the fatty acid enriched diets, but with these two groups both weighing significantly more than the control group

Table 2. Eight-week cumulative intake of mice on a standard diet (SD) or diets enriched with high oleic sunflower oil, (Unsaturated OiL-enriched Food, UOLF) or palm kernel oil (Saturated OiL-enriched Food, SOLF).

	SD	SOLF	UOLF	Statistics
Total food intake (g)	348.5 ± 3.1	159.4 ± 5.1***	162.4 ± 4.3***	$F(2,27)=659.8$; $p<0.001$
Total food intake (kcal)	1077.0 ± 9.5	864.1 ± 27.6***	862.4 ± 23.4***	$F(2,27)=32.51$; $p<0.001$
Total carbohydrate intake (g)	149.5 ± 1.3	42.8 ± 1.4***	43.9 ± 1.1***	$F(2,27)=2279$; $p<0.001$
Total protein intake (g)	73.2 ± 0.6	20.9±0.7***	21.0 ± 0.5***	$F(2,27)=2341$; $p<0.001$
Total ovoalbumin intake (g)	10.3 ± 0.1	2.6 ± 0.1***	2.8 ± 0.1***	$F(2,27)=2800$; $p<0.001$
Total lipid intake (g)	20.9 ± 0.2	65.6 ± 2.1***	66.3 ± 1.7***	$F(2,27)=273.3$; $p<0.001$
Total palmitic acid intake (g)	2.4 ± 0.0	9.7 ± 0.3***	4.8 ± 0.1***, ###	$F(2,27)=374$; $p<0.001$
Total oleic acid intake (g)	4.0 ± 0.0	16.0 ± 0.5***	50.8 ± 1.3***, ###	$F(2,27)=865.4$; $p<0.001$
Total lauric acid intake (g)	ND	31.6 ± 1.0	ND	---

Data are shown as mean ± SEM of 8–10 animals per group. ***$p < 0.001$, **$p <0.01$ and *$p <0.05$ vs the control group SD; ###$p <0.001$ *vs* SOLF group. ND (Not detected).

Table 3. Circulating metabolic parameters in mice on a standard diet (SD) or diets enriched with high oleic sunflower oil, (Unsaturated OiL-enriched Food, UOLF) or palm kernel oil (Saturated OiL-enriched Food, SOLF).

	SD	SOLF	UOLF	ANOVA
Glucose (mg/dL)	147.4 ± 4.8	194.1 ± 6.0**	187.4 ± 11.7**	$F_{(2,21)}$ 9.77, $p < 0.01$
Insulin (ng/mL)	1.4 ± 0.3	0.9 ± 0.3#	1.8 ± 0.3*	$F_{(2,20)}$ 7.911, $p < 0.01$
Triglycerides (mg/dL)	92.0 ± 10.9	82.6 ± 7.7	107.4 ± 17.5	ns
NEFA (mg/dL)	12.7 ± 1.5	20.2 ± 2.2*	13.9 ± 1.1	$F_{(2,21)}$ 5.94, $p < 0.01$
Leptin (ng/mL)	1.4 ± 0.4	3.1 ± 0.7#	6.6 ± 2.1*	$F_{(2,20)}$ 4.017, $p < 0.05$
TNFα (ng/mL)	3.2 ± 0.3	3.3 ± 0.5	4.7 ± 1.0	ns
IL6 (ng/mL)	5.1 ± 0.6	7.7 ± 1.6	6.2 ± 1.4	ns
aMCP1 (ng/mL)	31.8 ± 5.1	41.4 ± 5.4	44.6 ± 3.9	ns
PAI1 (ng/mL)	721.6 ± 56.7	1009 ± 175.6	1493 ± 71.6***	$F_{(2,20)}$ 10.7, $p < 0.001$

Values are means ± S.E.M. (n = 7 for the control and UOLF groups, n = 8 for the SOLF group). *$P < 0.05$ and **$P < 0.01$, compared to their respective controls. #$P < 0.05$ compared to UOLF. ns = non-significant.

(Figure 1D). There was an increase in the weight of the subcutaneous WAT depot (SD: 0.297 ± 0.017 g, SOLF: 0.536 ± 0.063 g, UOLF: 0.747 ± 0.086 g; $F_{(2,27)}$ 13.74; $p < 0.001$) and perigonadal WAT (SD: 0.407 ± 0.028 g, SOLF 0.933 ± 0.145 g, UOLF: 1.220 ± 0.111 g; $F_{(2,20)}$ 14.76; $p < 0.001$) in both SOLF and UOLF mice with no significant difference between these two groups. In contrast, although the mean weight of the mesenteric WAT depot increased with the high fatty acid diets, this did not reach statistical significance (SD 0.163 ± 0.033 g, SOLF: 0.213 ± 0.040, UOLF: 0.297 ± 0.490).

SOLF and UOLF diet effects on circulating metabolic factors

Glucose levels were elevated by both HFDs (Table 3); in contrast insulin levels were only elevated in mice on the UOLF diet such that they had higher levels than both controls and SOLF mice. Although triglyceride levels were not modified, NEFA levels were elevated after ingesting the SOLF diet. Leptin levels were increased by both fatty acid-enriched diet compared to the chow diet, with this increase being greater in the UOLF group such that their levels were significantly higher than those of SOLF mice. No changes in TNFα, IL6 or MCP1 levels were observed, while PAI1 increased significantly in the UOLF groups (Table 3).

Leptin expression in VAT and SCAT

Leptin mRNA levels in VAT increased with increased fat intake with this reaching statistical significance on the UOLF diet ($F_{(2,17)} = 4.263$, $p < 0.05$; Figure 2A). In SCAT, although leptin expression levels increased in response to both HFDs, these changes did not reach statistical significance (Figure 2B).

SOLF and UOLF induced changes in the hypothalamus

Both SOLF and UOLF intake decreased NPY mRNA levels in the hypothalamus ($F_{(2,21)}$ 11.5, $p < 0.001$; Figure 3A). Although there was also a decrease in AgRP mRNA levels, this did not reach significance in either group (Figure 3B). The mRNA levels of POMC increased in response to both diets ($F_{(2,21)} = 5.7$, $p < 0.005$; Figure 3C). Although modifications in circulating leptin levels were observed, no changes in

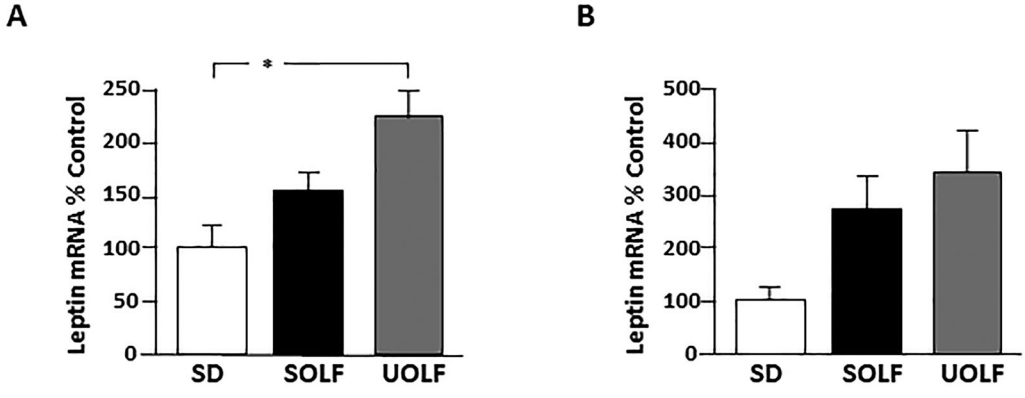


Figure 2. Relative leptin mRNA levels in visceral (A) and subcutaneous (B) adipose tissue in mice on a standard diet (SD) or diets enriched with high oleic sunflower oil, (Unsaturated OiL-enriched Food, UOLF) or palm kernel oil (Saturated OiL-enriched Food, SOLF). Data are shown as mean ± SEM of 8–10 animals per group. *$p < 0.05$.

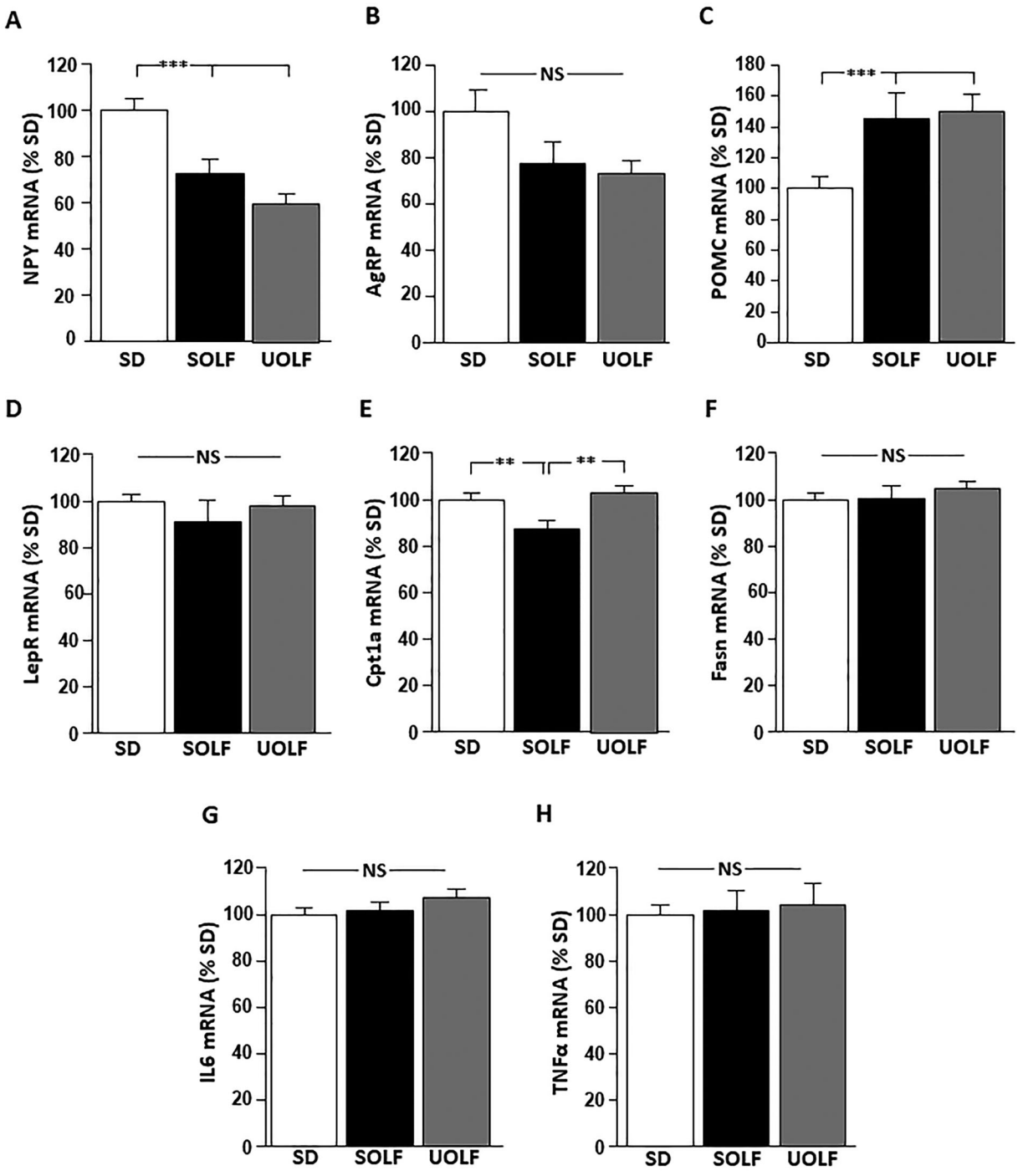


Figure 3. Relative hypothalamic leptin mRNA levels of neuropeptide Y (NPY; A) Agouti-related peptide (AgRP; B), proopiomelanocortin (POMC; C), leptin receptor (LepR; D), carnitine palmitoyltransferase 1A (CPT1a; E), fatty acid synthetase (Fasn; F), interleukin 6 (IL6; G), and tumor necrotizing factor α (TNF α; H) in mice on a standard diet (SD) or diets enriched with high oleic sunflower oil, (Unsaturated OiL-enriched Food, UOLF) or palm kernel oil (Saturated OiL-enriched Food, SOLF). Data are shown as mean ± SEM of 8–10 animals per group. $^{**}p < 0.01$, $^{***}p < 0.001$, NS = non-significant.

hypothalamic mRNA levels of its receptor were found (Figure 3D).

When analyzing factors involved with fatty acid metabolism, a decrease in Cpt1a was observed in the hypothalamus of mice eating a SOLF diet ($F_{(2,21)}$ 6.7, $p < 0.005$; Figure 3E). However, no modifications in Fasn mRNA (Figure 3F) levels were observed. Likewise, the expression of the cytokines IL6 (Figure 3G) and TNFα (Figure 3H) were not modified.

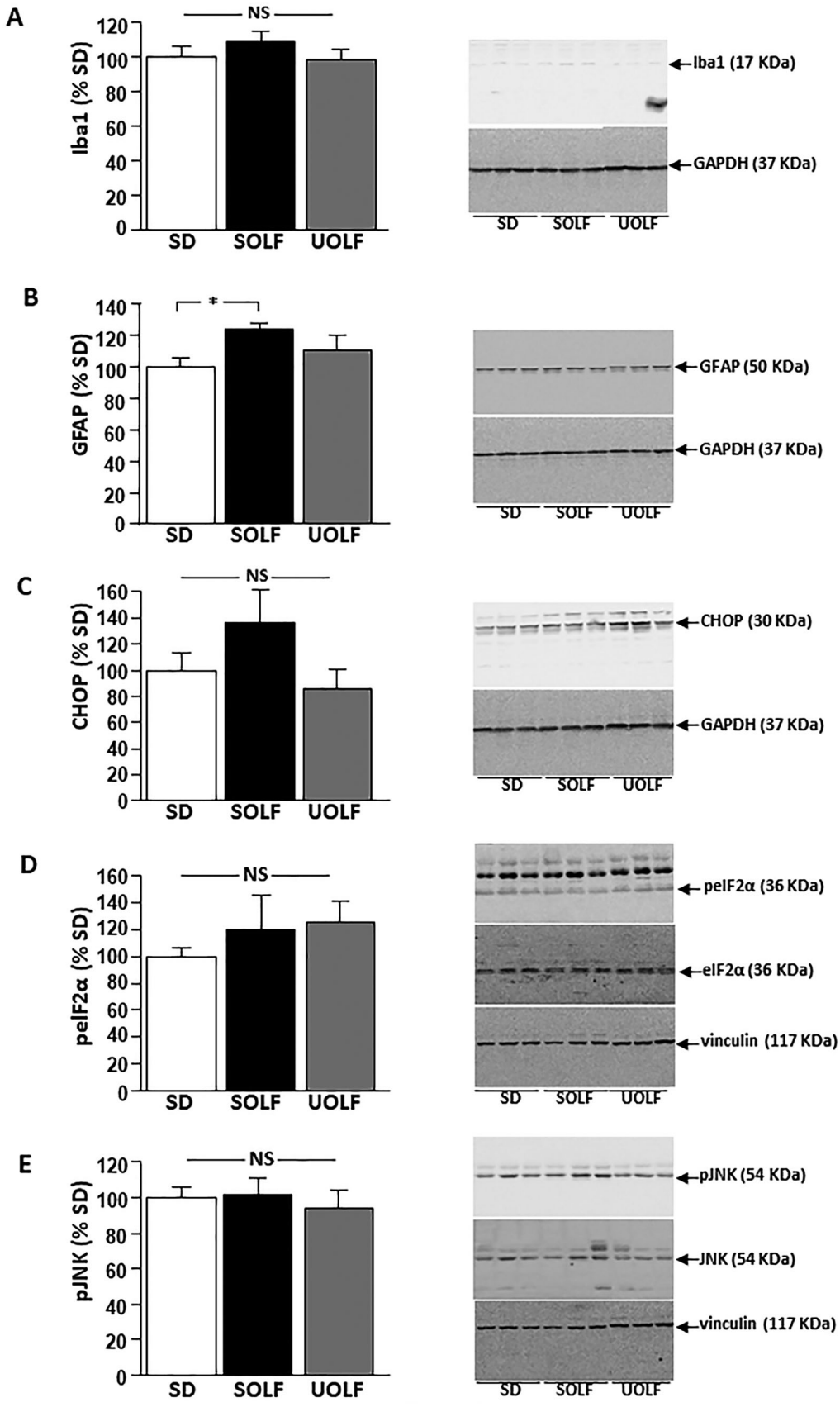


Figure 4. Relative protein levels of ionized calcium-binding adaptor molecule 1 (Iba1; A), fibrillary acidic protein (GFAP; B), CCAAT-enhancer-binding protein homologous protein (CHOP; C), elf2α (D) and pJNK (E) in mice on a standard diet (SD) or diets enriched with high oleic sunflower oil, (Unsaturated OiL-enriched Food, UOLF) or palm kernel oil (Saturated OiL-enriched Food, SOLF). Representative blots are shown. Data are shown as mean ± SEM of 6 animals per group. *$p < 0.05$, NS = non-significant.

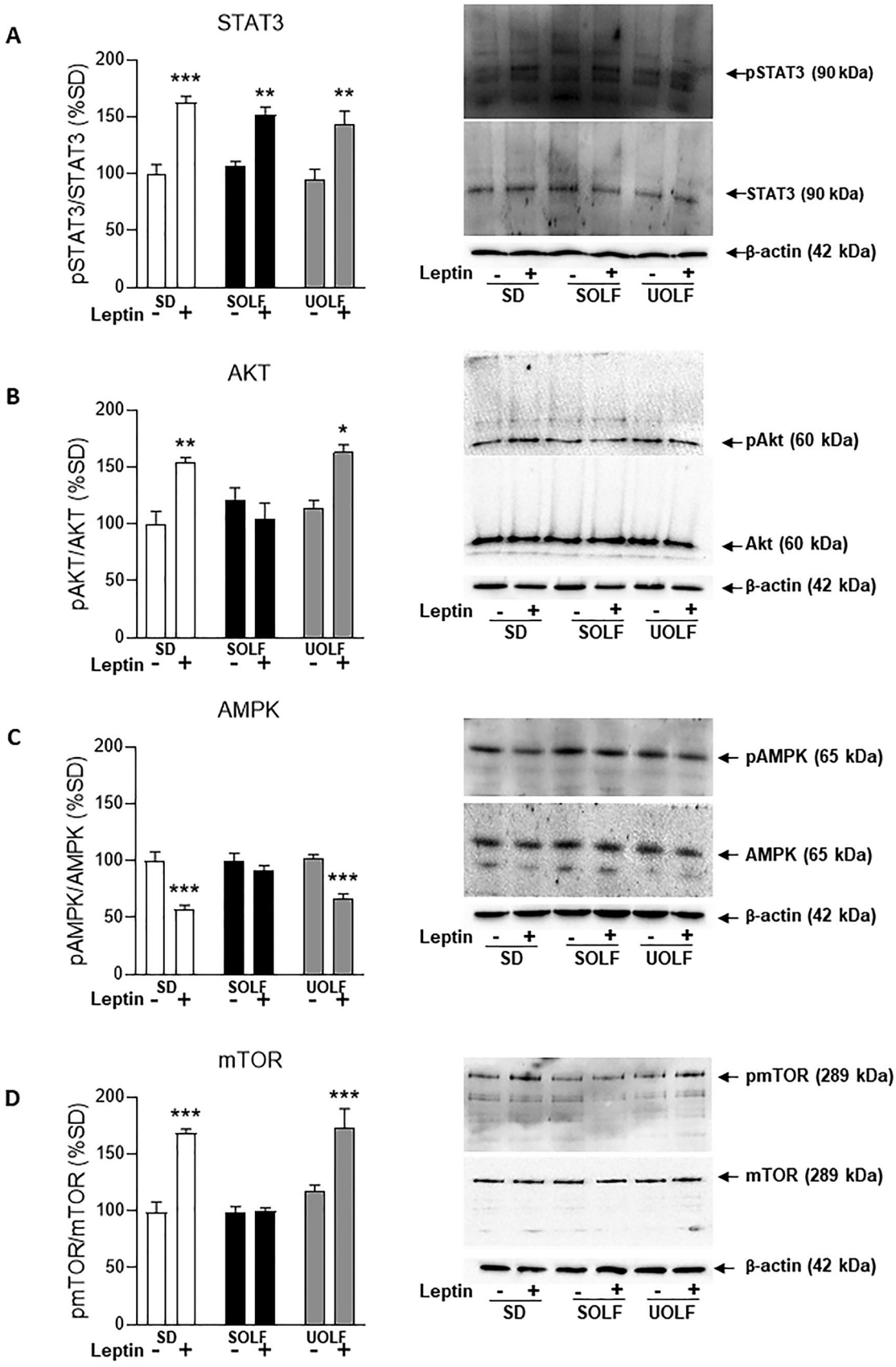


Figure 5. Relative protein levels of phosphorylated (p) pSTAT3 (A), pAKT (B), pAMPK (C), and pmTOR (D) in mice on a standard diet (SD) or diets enriched with high oleic sunflower oil, (Unsaturated OiL-*e*nriched Food, UOLF) or palm kernel oil (Saturated OiL-enriched Food, SOLF) after receiving a subcutaneous injection of vehicle (+) or leptin (+). Representative blots are shown. Data are shown as mean ± SEM of *6* animals per group. *$p < 0.05$, NS = non-significant

Protein levels of Iba1 and GFAP were measured to determine if microglia and astrocytes, respectively, were affected. Although Iba1 levels were not significantly modified (Figure 4A), GFAP levels were increased ($F_{(2,25)}$ 3.54, $p < 0.05$; Figure 4B) in mice on the saturated fatty acid diet. We found no modification in the protein levels of CHOP, elf2α, or pJNK (Figures 4C-E), proteins involved in endoplasmic reticular stress and inflammation.

SOLF but not UOLF modified the response to leptin in the hypothalamus

Exogenous leptin treatment stimulated hypothalamic pSTAT3 levels ($F_{(1,34)}$ = 62.01, $p < 0.001$; Figure 5A) regardless of the type of diet that the mice consumed. In contrast, the effect of leptin on pAKT (Figure 5B; $F_{(1,34)} = 13.45$, $p < 0.001$), pmTOR (Figure 5C; $F_{(1,34)}$ 34.59, $p < 0.001$), and pAMPK (Figure 5D; $F_{(1,34)}$ 40.62; $p < 0.001$) was dependent on dietary intake ($F_{(2,34)}$ 3.61, $p < 0.05$; $F_{(2,34)}$ 15.69, $p < 0.001$; $F_{(2,34)}$ 4.90, $p < 0.05$ for the interactions leptin x type of diet for pAKT, pmTOR and pAMPK, respectively). Leptin stimulated the phosphorylation of AKT and mTOR and decreased pAMPK levels in SD and UOLF mice, with no effect in SOLF mice.

Discussion

The results presented here clearly demonstrate that caloric intake is not the sole underlying factor in determining weight gain. Throughout the duration of this study there were no significant differences between the experimental groups in total energy intake; however, mice on the HFDs gained significantly more weight than those on the standard chow diet. These mice rapidly adjusted their energy intake to control levels after being subjected to the fatty acid enriched/high energy diets and maintained this situation throughout the 8 weeks of the study. The observed decrease in the expression of hypothalamic orexigenic neuropeptides (i.e. NPY and AgRP) and the increase in the expression of POMC, which is a precursor for anorexigenic neuropeptides, indicates a readjustment of the hypothalamic metabolic control mechanisms to reduce energy intake. Thus, this adjustment of neuropeptide balance could be to counterbalance the drive to overeat palatable high-energy foods and suggests that the homeostatic control system continues to function after consumption of these diets, at least up to 8 weeks. It should also be noted that these mice were not given a choice of food, which may also influence the normalization of caloric intake [24].

The increase in weight gain, despite a similar energy intake, could be explained by a decrease in energy expenditure in response to the HFDs. Although not analyzed here, decreased energy expenditure in response to HFD is not supported by studies showing that HFD intake actually stimulates thermogenesis in brown adipose tissue or others indicating that there is no effect on markers of thermogenesis in this tissue [25–27]. Diet composition modifies the gut microbiota, and this has been associated with changes in body weight and thermogenesis [28]. In addition, HFD intake modifies circadian rhythms including food intake patterns, and this increases weight gain even if energy intake is not increased [29] and with possible fatty acid-specific effects on circadian rhythms occurring at the hypothalamic level [30, 31]. Thus, further studies are necessary to determine the mechanisms underlying the increased weight gain in response to these specific fatty acid-enriched diets.

Although there were no differences between the two different fatty acid-enriched diets in their effects on weight gain or energy intake, their effects on systemic metabolism differed. An elevation in circulating NEFA levels was only observed in mice ingesting the SOLF, which is consistent with reports showing that NEFA levels are increased in HFD-induced obesity, but not high carbohydrate-induced obesity, when employing commercially available HFDs rich in saturated fatty acids [32]. Elevated NEFA levels are associated with increased risk of insulin resistance and type 2 diabetes [33]. However, although glucose levels were increased in both UOLF and SOLF mice, insulin levels were only elevated in the group consuming the unsaturated fatty acid diet. In a previous study, plasma insulin levels were found to be lower in fasted UOLF compared to fasted SOLF mice [21]. This observation might be related to differences between SOLF and UOLF mice in circadian feeding behavior and future studies are required to test this hypothesis, as well as to better characterize the ability of these animals to manage glucose although it is clear here that an increased intake of saturated versus unsaturated fatty acids differentially affects glucose metabolism. Indeed, a differential response to saturated and unsaturated fats on glucose-stimulated insulin secretion has been previously reported [34].

Adipose tissue produces high levels of PAI1 and knockout mice for PAI1 are more resistant to HFD-induced obesity with this effect possibly being mediated through PAI-1 modulation of hypothalamic leptin resistance [35]. Here circulating levels of PAI-1 were elevated in both groups ingesting fatty acid-enriched diets, which is in accordance with their increased weight gain. In contrast, only SOLF mice were found to exhibit attenuated responses to leptin at the hypothalamic level,

suggesting that other factors are also involved in the development of this phenomenon. Indeed, both leptin production and signaling were differentially modulated by the fatty acid-enriched diets employed here. Circulating leptin levels were increased by both diets, but this rise was significantly greater in mice on the unsaturated fatty acid diet. These modifications in serum leptin levels were coincident with a significant rise in leptin mRNA levels in the adipose tissue of UOLF mice, while this increase was not significant in SOLF animals. Increased circulating leptin levels in obesity are due to increased fat mass and/or increased synthesis of this adipokine. Although we found that the weights of the WAT deposits were not statistically different between SOLF and UOLF mice, there was a tendency for UOLF mice to have higher levels of adipose tissue compared to SOLF mice and this most likely would be more apparent with more precise measurements. Thus, it appears that plasma leptin levels are higher in UOLF mice in accordance with higher *Lep* expression levels, as well as possibly the amount of WAT.

Leptin feeds back at the hypothalamic level to relay information regarding systemic metabolic status and if the transport of and/or sensitivity to this hormone are not affected, increased leptin levels should inhibit food intake. Here energy intake in both HFD groups was normalized, but higher levels of circulating leptin and increased POMC and decreased NPY expression in the hypothalamus were found, thus suggesting a new *statu quo*. In fact, STAT3 signaling, which has been shown to account for NPY/AGRP repression independently of the functionality of other pathways coupled to LepR [36], remains operative in both SOLF and UOLF mice. However, alternative leptin signaling pathways are affected in SOLF mice and this does not appear to be directly, or at least solely, associated to the hyperleptinemia as they have lower leptin levels than UOLF mice. This leads one to speculate that saturated FAs may have a 'direct' effect on LepR responsiveness or possibly modulate central leptin levels by altering transport through the blood brain barrier [37], although this later mechanism has been challenged [38]. It is also possible that transport within the hypothalamus is affected, with this potentially involving glial cells [39]. Indeed, direct activation of both the PI3 K/AMPK/Akt and PI3 K/ERK1/2 pathways through membrane effects of palmitic acid has been reported in skeletal muscle [40] and palmitic acid inhibits insulin-dependent activation of Akt. Mice on the SOLF diet had higher levels of palmitic acid than those on the UOLF diet and thus, it is possible that direct effects of palmitic acid on these intracellular signaling mechanisms could be involved in the different responses to acute leptin administration observed between UOLF and SOLF mice. However, other components of the diet, such as higher lauric acid levels in the SOLF diet, could also be involved. Indeed, medium-chain triglycerides, very abundant in SOLF, have been shown to modulate the AKT, AMPK and mTOR pathways outside of the CNS [41, 42].

No difference in the hypothalamic response to exogenous leptin was observed between UOLF mice and controls, but the ability of leptin to activate AKT and mTOR and to inhibit AMPK was hampered in SOLF mice. Indeed, leptin is known to reduce AMPK activity and increase mTOR activation in the hypothalamus with these changes participating in leptin's effects on metabolic neuropeptide expression and metabolic control, as well as glucose metabolism [43, 44]. This indicates that long-term intake of these two diets differentially modulates central leptin signaling, even though the modifications in anorexigenic and orexigenic neuropeptides expression was similar. One caveat that should be taken into consideration is that the leptin response was analyzed in the entire hypothalamus, which does not take into account the fact that different cell types might be differentially affected. Here it appears that POMC and NPY/AgRP neurons continue to respond to leptin, or at least the observed modifications in these neuropeptides are what might be expected in situations of high leptin levels, and this could be related to the fact that activation of STAT3 in response to leptin appears to remain intact. It should be noted that basal activation of these pathways did not differ between the experimental groups, even though leptin levels were higher in the HFD groups. This could thus indicate a potential difference in leptin sensitivity in these groups and that the response to higher levels or fluctuations in this adipokine is more affected in the SOLF group. More long-term exposure to these diets could help to clarify the time-dependent effects on this system.

Palmitic acid has been considered the culprit of many of the noxious effects of our diet with many studies focusing on this fatty acid. However, in the palm kernel enriched diet, lauric acid levels were considerably elevated. Diets rich in lauric acid can induce excess weight gain, but they appear to be less inflammatory or less likely to promote insulin resistance than those rich in palmitic acid [45, 46] and to possibly even improve insulin resistance in some cell types [47]. Less is known regarding the central effects of lauric acid, but it has been reported to be less pro-inflammatory than palmitic acid in the mHypoE-N42 hypothalamic cell line [48]. Thus, as the metabolic effects of the palm kernel-enriched diet are the combination of the different nutritional components, some adverse effects of

palmitic acid could be buffered by the high content of lauric acid.

Hypothalamic inflammation and gliosis have been associated with the development of insulin and leptin resistance [7, 49] and here the intake of increased levels of saturated fatty acids was associated with higher GFAP levels, which is commonly used as an indication of astrogliosis. This agrees with studies indicating that astrocytes are more susceptible to activation by saturated fatty acids as opposed to unsaturated fatty acids [17] and this increased astrogliosis could be involved with the modifications in leptin sensitivity observed in the SOLF mice. Furthermore, astrocytes are the main site for fatty acid oxidation in the brain [50], and these glial cells may be less capable of oxidizing palmitic acid compared to oleic acid. Indeed, in studies of astrocytes in vitro, we previously reported that oleic acid stimulates the expression of CPT1a, an enzyme necessary for transport of fatty acids into the mitochondria to be metabolized, while palmitic acid does not [18]. Here, hypothalamic expression of CPT1a was decreased in the hypothalamus of SOLF mice. Thus, this could result in accumulation of palmitic acid in the hypothalamus, as previously reported in response to commercial HFDs [51], causing astrogliosis. In contrast, no indications of hypothalamic microgliosis, ER stress or activation of inflammatory pathways were observed with either fatty acid-enriched diet. Although consumption of HFDs do not always result in hypothalamic inflammation, which may be due to the age of the animal, duration of the diet, early developmental effects and sex, and dietary composition [51–54], this observation deserves further investigation.

There is a clear difference in the response to the two fatty acid enriched diets used in these studies and this is most likely due to their different fatty acid compositions as other components were very similar. However, identification of the nutrients responsible for the different metabolic response to these diets compared to that observed in chow fed mice is less direct. Not only is the fatty acid content different, but also that of proteins and carbohydrates, as well as of fiber and some vitamins and minerals, all of which could affect metabolism. Thus, one can only conclude that these diets enriched in fatty acids induce weight gain and other metabolic changes compared to a standard chow diet, which is assumed to be a healthy diet for these animals. Indeed, the response to high fat diet intake depends on the control diet used for comparison [55].

The fact that only males were included in the studies presented here must also be taken into consideration. Males and females have different propensities to develop obesity and its complications, and they respond differently to HFD intake, including differences in energy intake, weight gain, adipose tissue accrual and distribution, modifications in glucose tolerance, the development of gliosis/hypothalamic inflammation, etc. [51, 56–58]. Moreover, hypothalamic cells derived from males and females respond differently to PA in vitro [51, 59]. Thus, one might predict that there would be sex differences in the response to these diets enriched in specific fatty acids, although this remains to be demonstrated.

In conclusion, dietary composition is of utmost importance for determining long-term health effects. Excess energy intake over time can clearly result in obesity; but here excess weight gain was observed even though overall energy intake was not increased. Moreover, the fatty acid composition of the high fat diets differentially modulated systemic metabolism, with these effects possibly being due, at least in part to the metabolic response of hypothalamic astrocytes to different fatty acids. Thus, further studies to determine the central metabolic response to specific fatty acids will be of interest. Understanding how specific nutrients regulate metabolism are of utmost importance for developing healthy diets and for the possibility of further precision diets.

Acknowledgements

We would like to thank Maria Jimenez and Sandra Canelles for their excellent technical support.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research was funded by grants from the Spanish Ministry of Science and Innovation (BFU2017-82565-C21-R2 to J.A.C. and L.M.F., BFU2016-78556-R and PID2020-117422RB-C21 to M.R-G, and BFU2017-82565-C2-2-R to B.S. and M.S.-F.A.), Centro de Investigación Biomédica en Red Fisiopatología de Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III Fondo de Investigación Sanitaria and Fondos FEDER (J.A.C.). Fundación Universitaria San Pablo-CEU, and FEDER. J.F-F. is supported by a grant from FUSP-CEU

Data availability statement

All data are available upon request.

Notes on contributors

Jesús Fernández-Felipe is a doctoral student in the Faculty of Pharmacy, Universidad San Pablo-CEU, Madrid.

Maria Valencia-Avezuela is now a doctoral student in the Instituto de Investigaciones Científicas Alberto Sols, CSIC, Madrid. This work was part of her Master's Thesis at the Hospital Infantil Universitario Niño Jesús.

Beatriz Merino is professor in the Faculty of Pharmacy, Universidad San Pablo-CEU, Madrid.

Beatriz Somoza is professor in the Faculty of Pharmacy, Universidad San Pablo-CEU, Madrid.

Victoria Cano is professor in the Faculty of Pharmacy, Universidad San Pablo-CEU, Madrid.

Ana B. Sanz-Martos is a doctoral student in the Faculty of Pharmacy, Universidad San Pablo-CEU, Madrid.

Laura M. Frago is professor at the Faculty of Medicine, Universidad Autónoma de Madrid.

Maria S. Fernández-Alfonso is full professor in the Department of Pharmacy at the Universidad Complutense, Madrid.

Mariano Ruiz-Gayo is full professor at the Faculty of Pharmacy, Universidad San Pablo-CEU, Madrid.

Julie A. Chowen is Senior Researcher at the Hospital Infantil Universitario Niño Jesús, Madrid.

ORCID

Julie A. Chowen http://orcid.org/0000-0002-4770-2291

References

[1] Martin-Rodriguez E, et al. Comorbidity associated with obesity in a large population: The APNA study. Obes Res Clin Pract. 2015;9(5):435–447.
[2] Martos-Moreno GA, et al. Principles and pitfalls in the differential diagnosis and management of childhood obesities. Adv Nutr. 2014;5(3):299S–305S.
[3] Cheng D, et al. Metabolomic signature between Metabolically healthy overweight/obese and Metabolically unhealthy overweight/obese: A systematic review. Diabetes Metab Syndr Obes. 2021;14:991–1010.
[4] Duque AP, et al. Emerging concepts in metabolically healthy obesity. Am J Cardiovasc Dis. 2020;10(2):48–61.
[5] Estruch R, Ros E. The role of the Mediterranean diet on weight loss and obesity-related diseases. Rev Endocr Metab Disord. 2020;21(3):315–327.
[6] San-Cristobal R, et al. Contribution of macronutrients to obesity: implications for precision nutrition. Nat Rev Endocrinol. 2020;16(6):305–320.
[7] De Souza CT, et al. Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology. 2005;146(10):4192–9.
[8] Xu H, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest. 2003;112(12):1821–1830.
[9] Wu L, et al. Activation of invariant natural killer T cells by lipid excess promotes tissue inflammation, insulin resistance, and hepatic steatosis in obese mice. Proc Natl Acad Sci U S A. 2012;109(19):E1143–E1152.
[10] Thaler JP, et al. Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest. 2012;122(1):153–62.
[11] Horvath TL, et al. Synaptic input organization of the melanocortin system predicts diet-induced hypothalamic reactive gliosis and obesity. Proc Natl Acad Sci U S A. 2010;107(33):14875–80.
[12] Calegari VC, et al. Inflammation of the hypothalamus leads to defective pancreatic islet function. J Biol Chem. 2011;286(15):12870–80.
[13] Milanski M, et al. Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. J Neurosci. 2009;29(2):359–70.
[14] Valdearcos M, et al. Microglia dictate the impact of saturated fat consumption on hypothalamic inflammation and neuronal function. Cell Rep. 2014;9(6):2124–38.
[15] Gruber T, et al. Obesity-associated hyperleptinemia alters the gliovascular interface of the hypothalamus to promote hypertension. Cell Metab. 2021;33(6):1155–1170.
[16] Zhang Y, et al. Astrocytic process plasticity and IKKbeta/NF-kappaB in central control of blood glucose, blood pressure, and body weight. Cell Metab. 2017;25(5):1091–1102.
[17] Gupta S, et al. Saturated long-chain fatty acids activate inflammatory signaling in astrocytes. J Neurochem. 2012;120(6):1060–1071.
[18] Argente-Arizon P, et al. The hypothalamic inflammatory/gliosis response to neonatal overnutrition Is Sex and Age dependent. Endocrinology. 2018;159(1):368–387.
[19] Kilkenny C, et al. Animal research: reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol. 2010;160(7):1577–9.
[20] McGrath JC, et al. Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol. 2010;160(7):1573–6.
[21] Plaza A, et al. Potential role of leptin in cardiac steatosis induced by highly saturated Fat intake during adolescence. Mol Nutr Food Res. 2019;63(19):e1900110.
[22] Guzman-Ruiz R, et al. Sensitivity of cardiac carnitine palmitoyltransferase to malonyl-CoA is regulated by leptin: similarities with a model of endogenous hyperleptinemia. Endocrinology. 2010;151(3):1010–8.
[23] Frago LM, et al. Growth hormone (GH) and GH-releasing peptide-6 increase brain insulin-like growth factor-I expression and activate intracellular signaling pathways involved in neuroprotection. Endocrinology. 2002;143(10):4113–22.
[24] Slomp M, et al. Stressing the importance of choice: validity of a preclinical free-choice high-caloric diet paradigm to model behavioural, physiological and molecular adaptations during human diet-induced obesity and metabolic dysfunction. J Neuroendocrinol. 2019;31(5):e12718.
[25] Perez GS, et al. Does a high-fat diet-induced obesity model brown adipose tissue thermogenesis? A systematic review. Arch Med Sci. 2021;17(3):596–602.
[26] Kim KS, et al. Taurine stimulates thermoregulatory genes in brown Fat Tissue and muscle without an

influence on inguinal white Fat Tissue in a high-Fat diet-induced obese Mouse model. Foods. 2020;9(6):688.
[27] Zhang L, et al. Diet-induced adaptive thermogenesis requires neuropeptide FF receptor-2 signalling. Nat Commun. 2018;9(1):4722.
[28] Wang B, et al. A high-Fat diet increases Gut microbiota biodiversity and energy expenditure Due to nutrient difference. Nutrients. 2020;12(10).
[29] Hatori M, et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell Metab. 2012;15 (6):848–60.
[30] Greco JA, Oosterman JE, Belsham DD. Differential effects of omega-3 fatty acid docosahexaenoic acid and palmitate on the circadian transcriptional profile of clock genes in immortalized hypothalamic neurons. Am J Physiol Regul Integr Comp Physiol. 2014;307(8): R1049–60.
[31] Clemenzi MN, et al. Analysis of Western diet, palmitate and BMAL1 regulation of neuropeptide Y expression in the murine hypothalamus and BMAL1 knockout cell models. Mol Cell Endocrinol. 2020;507:110773.
[32] Dourmashkin JT, et al. Different forms of obesity as a function of diet composition. Int J Obes (Lond). 2005;29(11):1368–78.
[33] Bergman RN, Ader M. *Free fatty acids and pathogenesis of type 2 diabetes mellitus*. Trends Endocrinol Metab. 2000;11(9):351–6.
[34] Dobbins RL, et al. The composition of dietary fat directly influences glucose-stimulated insulin secretion in rats. Diabetes. 2002;51(6):1825–33.
[35] Hosaka S, et al. Inhibition of plasminogen activator inhibitor-1 activation suppresses high Fat diet-induced weight gain via alleviation of hypothalamic leptin resistance. Front Pharmacol. 2020;11:943.
[36] Morrison CD, et al. Leptin inhibits hypothalamic Npy and agrp gene expression via a mechanism that requires phosphatidylinositol 3-OH-kinase signaling. Am J Physiol Endocrinol Metab. 2005;289 (6):E1051–7.
[37] Burguera B, et al. Obesity is associated with a decreased leptin transport across the blood-brain barrier in rats. Diabetes. 2000;49(7):1219–23.
[38] Kleinert M, et al. Time-resolved hypothalamic open flow micro-perfusion reveals normal leptin transport across the blood-brain barrier in leptin resistant mice. Mol Metab. 2018;13:77–82.
[39] Balland E, et al. Hypothalamic tanycytes are an ERK-gated conduit for leptin into the brain. Cell Metab. 2014;19(2):293–301.
[40] Pu J, et al. Palmitic acid acutely stimulates glucose uptake via activation of Akt and ERK1/2 in skeletal muscle cells. J Lipid Res. 2011;52(7):1319–27.
[41] Wang ME, et al. Increasing dietary medium-chain fatty acid ratio mitigates high-fat diet-induced Non-Alcoholic steatohepatitis by regulating autophagy. Sci Rep. 2017;7(1):13999.
[42] Rial SA, et al. Hexanoic, octanoic and decanoic acids promote basal and insulin-induced phosphorylation of the Akt-mTOR axis and a balanced lipid metabolism in the HepG2 hepatoma Cell line. Molecules. 2018;23 (9):2315–2331.
[43] Minokoshi Y, et al. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature. 2004;428(6982):569–74.
[44] Hu F, Xu Y, Liu F. Hypothalamic roles of mTOR complex I: integration of nutrient and hormone signals to regulate energy homeostasis. Am J Physiol Endocrinol Metab. 2016;310(11):E994–E1002.
[45] Saraswathi V, et al. Lauric acid versus palmitic acid: effects on adipose tissue inflammation, insulin resistance, and Non-Alcoholic fatty liver disease in obesity. Biology (Basel). 2020;9(11):346.
[46] DiNicolantonio JJ, O'Keefe JH. Good fats versus Bad fats: A comparison of fatty acids in the promotion of insulin resistance, inflammation, and obesity. Mo Med. 2017;114(4):303–307.
[47] Tham YY, et al. Lauric acid alleviates insulin resistance by improving mitochondrial biogenesis in THP-1 macrophages. Mol Biol Rep. 2020;47(12):9595–9607.
[48] Sergi D, et al. Palmitic acid triggers inflammatory responses in N42 cultured hypothalamic cells partially via ceramide synthesis but not via TLR4. Nutr Neurosci. 2020;23(4):321–334.
[49] Douglass JD, et al. Astrocyte IKKbeta/NF-kappaB signaling is required for diet-induced obesity and hypothalamic inflammation. Mol Metab. 2017;6(4):366–373.
[50] Rose J, et al. Mitochondrial metabolism in astrocytes regulates brain bioenergetics, neurotransmission and redox balance. Front Neurosci. 2020;14:536682.
[51] Morselli E, et al. A sexually dimorphic hypothalamic response to chronic high-fat diet consumption. Int J Obes (Lond. 2015;40(2):206–209.
[52] Sanchez-Garrido MA, et al. Metabolic and gonadotropic impact of sequential obesogenic insults in the female: influence of the loss of ovarian secretion. Endocrinology. 2015;156(8):2984–98.
[53] Mela V, et al. Interaction between neonatal maternal deprivation and serum leptin levels on metabolism, pubertal development, and sexual behavior in male and female rats. Biol Sex Differ. 2016;7:2.
[54] Freire-Regatillo A, et al. Sex differences in the peripubertal response to a short-term, high-fat diet intake. J Neuroendocrinol. 2020;32(1):e12756.
[55] Guerra-Cantera S, et al. Short-Term diet induced changes in the central and circulating IGF systems Are Sex specific. Front Endocrinol (Lausanne. 2020;11:513.
[56] Chowen JA, Freire-Regatillo A, Argente J. Neurobiological characteristics underlying metabolic differences between males and females. Prog Neurobiol. 2019;176:18–32.
[57] Fuente-Martin E, et al. Estrogen, astrocytes and the neuroendocrine control of metabolism. Rev Endocr Metab Disord. 2013;14(4):331–8.
[58] Fuente-Martin E, et al. Sex differences in adipose tissue: It is not only a question of quantity and distribution. Adipocyte. 2013;2(3):128–34.
[59] Morselli E, et al. Hypothalamic PGC-1alpha protects against high-fat diet exposure by regulating ERalpha. Cell Rep. 2014;9(2):633–45.

PUBLICACIÓN 3: 'EFFECT OF LAURIC VS. OLEIC ACID-ENRICHED DIETS ON LEPTIN AUTOPARACRINE SIGNALLING IN MALE MICE'

Fernández-Felipe J, Plaza A, Domínguez G, Pérez-Castells J, Cano V, Cioni F, Del Olmo N, Ruiz-Gayo M, Merino B. Effect of Lauric vs. Oleic Acid-Enriched Diets on Leptin Autoparacrine Signalling in Male Mice. Biomedicines. 2022 Aug 2;10(8):1864

https://doi.org/10.3390/ biomedicines10081864

RESUMEN

Las dietas enriquecidas en ácido láurico (SOLF) no promueven la producción de leptina a pesar de expandir el tejido adiposo blanco. Este estudio tiene como **objetivo** identificar la influencia de SOLF frente a una dieta enriquecida en ácido oleico (UOLF) sobre el efecto autocrino de la leptina. El estudio se llevó a cabo en ratones macho de 5 semanas de edad que consumieron una dieta control, SOLF o UOLF durante 8 semanas. Se analizaron en el tejido adiposo blanco 1) la fosforilación de las proteínas implicadas en la señalización acoplada al receptor de leptina, 2) la distribución de tamaños de los adipocitos y 3) el contenido en ácidos grasos y la expresión génica de genes implicados en la diferenciación de adipocitos, inflamación y fibrosis. La dieta SOLF promueve la fosforilación basal de las proteínas estudiadas pero redujo la capacidad de la leptina de aumentar su fosforilación. Al contrario, los animales UOLF presentaron niveles basales y la respuesta a leptina similar a los animales control. Tanto SOLF como UOLF afectaron de forma similar a la distribución de tamaños de adipocitos y a la expresión de genes relacionados con la adipogenesis e inflamación. La composición del tejido adiposo blanco fue diferente entre grupos, encontrándose en las muestras de SOLF principalmente los ácidos láurico, palmítico y mirístico (> 48 % p/p) y en las UOLF más de un 80 % p/p de ácido oleico. En conclusión, la dieta SOLF parece ser más perjudicial que la dieta UOLF sobre las acciones autocrinas de la leptina, lo cual puede tener un impacto sobre la inflamación del tejido adiposo. El efecto de SOLF y UOLF sobre la

composición del tejido adiposo podría afectar a sus propiedades biofísicas, las cuales son capaces de condicionar la señalización del receptor de leptina.

biomedicines

MDPI

Article

Effect of Lauric vs. Oleic Acid-Enriched Diets on Leptin Autoparacrine Signalling in Male Mice

Jesús Fernández-Felipe [1,†], Adrián Plaza [1,2,†], Gema Domínguez [3], Javier Pérez-Castells [3], Victoria Cano [1], Francesco Cioni [1], Nuria Del Olmo [4], Mariano Ruiz-Gayo [1,*] and Beatriz Merino [1,*]

[1] Department of Health and Pharmaceutical Sciences, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, 28660 Madrid, Spain; jesus.fernandezfelipe@ceu.es (J.F.-F.); adrian.plaza@imdea.org (A.P.); victoria.cano@ceu.es (V.C.); cioni.1694862@studenti.uniroma1.it (F.C.)
[2] Laboratory of Bioactive Products and Metabolic Syndrome (BIOPROMET), IMDEA Food Institute, 28049 Madrid, Spain
[3] Department of Chemistry and Biochemistry, Facultad de Farmacia, Universidad CEU-San Pablo, CEU Universities, 28660 Madrid, Spain; gdommar@ceu.es (G.D.); jpercas@ceu.es (J.P.-C.)
[4] Departament of Psychobiology, Facultad de Psicología, Universidad Nacional de Educación a Distancia, 28040 Madrid, Spain; nuriadelolmo@psi.uned.es
* Correspondence: ruigayo@ceu.es (M.R.-G.); merpala.fcex@ceu.es (B.M.)
† These authors contributed equally to this work.

Abstract: High-fat diets enriched with lauric acid (SOLF) do not enhance leptin production despite expanding white adipose tissue (WAT). Our study aimed at identifying the influence of SOLF vs. oleic acid-enriched diets (UOLF) on the autoparacrine effect of leptin and was carried out on eight-week-old mice consuming control chow, UOLF or SOLF. Phosphorylation of kinases integral to leptin receptor (LepR) signalling pathways (^{705}Tyr-STAT3, ^{473}Ser-Akt, ^{172}Thr-AMPK), adipocyte-size distribution, fatty acid content, and gene expression were analyzed in WAT. SOLF enhanced basal levels of phosphorylated proteins but reduced the ability of leptin to enhance kinase phosphorylation. In contrast, UOLF failed to increase basal levels of phosphorylated proteins and did not modify the effect of leptin. Both SOLF and UOLF similarly affected adipocyte-size distribution, and the expression of genes related with adipogenesis and inflammation. WAT composition was different between groups, with SOLF samples mostly containing palmitic, myristic and lauric acids (>48% *w*/*w*) and UOLF WAT containing more than 80% (*w*/*w*) of oleic acid. In conclusion, SOLF appears to be more detrimental than UOLF to the autoparacrine leptin actions, which may have an impact on WAT inflammation. The effect of SOLF and UOLF on WAT composition may affect WAT biophysical properties, which are able to condition LepR signaling.

Keywords: adipose tissue hypertrophy; leptin resistance; monounsaturated fatty acids; perigonadal adipose tissue; saturated fatty acids; subcutaneous adipose tissue; visceral adipose tissue

Citation: Fernández-Felipe, J.; Plaza, A.; Domínguez, G.; Pérez-Castells, J.; Cano, V.; Cioni, F.; Del Olmo, N.; Ruiz-Gayo, M.; Merino, B. Effect of Lauric vs. Oleic Acid-Enriched Diets on Leptin Autoparacrine Signalling in Male Mice. *Biomedicines* **2022**, *10*, 1864. https://doi.org/10.3390/biomedicines10081864

Academic Editor: Arleta Drozd

Received: 29 June 2022
Accepted: 29 July 2022
Published: 2 August 2022

1. Introduction

White adipose tissue (WAT) plays a crucial role in body energy homeostasis due to its metabolic plasticity and endocrine function. Negative energy balance promotes lipolytic mechanisms leading to fatty acid (FA) and glycerol release, necessary to fulfil energy needs in other tissues; in contrast, during periods of overfeeding, WAT stores energy in the form of triglycerides (TG), useful for periods of food shortage. This mechanism contributes to limit ectopic accumulation of lipids in non-adipose tissues and reduces the risk of lipotoxicity [1,2]. Metabolic activity of WAT is regulated both by neuronal and endocrine signals, including adipokines [3–5], which are pivotal inorchestrating energy balance regulation by modulating food intake, WAT plasticity, and energy metabolism.

Long periods of elevated intake of simple carbohydrates and/or fat trigger adaptive remodelling of WAT, characterized by the increase in adipocyte size (hypertrophy) and

proliferation (hyperplasia), affecting both subcutaneous (Sc-WAT) and visceral WAT (Vis-WAT). These changes are accompanied by a concomitant increase in leptin production [6,7]. In addition to its endocrine role, pivotal in regulating food intake and fat catabolism, physiological concentrations of leptin are endowed with an autocrine-paracrine function that promotes adipogenesis by a mechanism involving PPARγ [8]. In contrast, hyperleptinemia associated with diet-induced obesity (DIO) has been shown to inhibit adipocyte differentiation and to promote WAT dysfunction [3,9].

The detrimental effect of hyperleptinemia is associated with the development of leptin resistance in many tissues/organs [10–15] able to trigger WAT hypertrophy [16] and ectopic accumulation of lipids [17–19]. In fact, fat storage in adipocytes has been shown to require the inactivation of leptin paracrine activity [16]. Despite all this research demonstrating the consequences of hyperleptinemia and leptin resistance for WAT function [16,19–21], an adequate characterization of the WAT leptin receptor (LepR) functionality under conditions of DIO is lacking. Particularly, the specific influence that saturated vs. monounsaturated fat may have on WAT LepR responsiveness has not been sufficiently investigated. In the same vein, the effect of these types of fat on WAT morphology remains poorly characterized. It should be highlighted that diets enriched with saturated fat (SOLF), and particularly with lauric acid, do not enhance leptin production despite expanding WAT.

Our hypothesis is that saturated and monounsaturated fat differently affect the autoparacrine function of leptin. The aim of the current study is to investigate the influence of saturated and monounsaturated fat on LepR functionality in both Sc and Vis-WAT. For this purpose, we analysed the responsiveness of adipose LepR to exogenous leptin in mice that consumed (eight weeks) sugar-free diets enriched either in monounsaturated (high-oleic sunflower oil, UOLF) or saturated fat (palm kernel oil, SOLF). Moreover, we characterized the effect of these diets on WAT remodelling, which is known to be a leptin-sensitive process.

2. Material and Methods

2.1. Animals and Diets

Assays were carried out in C57BL male mice (Charles River, Écully, France) that started to consume the experimental diets on postnatal day 35 (p35). Experimental procedures were carried out in accordance with the European Union Laboratory Animal Care Rules (86/609/EEC) and were approved by the Animal Research Committee of San Pablo CEU University (PCD Ref. PROEX-017/18. Approval date, 23 March 2017). Four-week-old animals were single housed under a 12-h light/12-h dark cycle, in a temperature-controlled room (22 °C) with water and rodent chow available *ad libitum*. After one week, animals with similar average body weight (BW) were randomly assigned to one of three experimental groups that were fed (8 weeks) either standard chow (SD, Teklad global 2018, Harlan Laboratories, Indianapolis, IN, USA), SD enriched with 40% high-oleic sunflower oil (Unsaturated OiL-enriched Food, UOLF), or SD enriched with 40% palm kernel oil (Saturated OiL-enriched Food, SOLF) (see diets' composition in Table S1, Supplementary Materials). UOLF and SOLF provided about 70% energy from fat (vs. 18% in chow), with oleic acid (18.1 cis 9; 31%) and lauric acid (12:0)/palmitic acid (16:0) (20%/6%) being the most abundant FA in UOLF and SOLF, respectively. BW was monitored once a week. WAT morphology and composition, and gene expression were analysed in samples obtained from animals used in a previous investigation [12]. A second group of mice was employed to assess leptin resistance in WAT. Group sizes (6–7 animals per group) were calculated based on previous studies by our group and an expected 10% increase of BW after 8 weeks of dietary treatment, a statistical power >85% and $p < 0.05$.

2.2. Determination of Subcutaneous and Visceral White Adipose Tissue Composition

Lipid composition was determined by means of ^{13}C nuclear magnetic resonance (^{13}C-NMR) of an FA pool obtained by saponification of total WAT lipids. For saponification, total Sc or Vis-WAT lipids, extracted by using the Folch method modified by Herrera and Ayanz [22], 1972 (100–130 mg), were dissolved in 0.7 mL of tetrahydrofuran and mixed with 0.7 mL of 1 M NaOH. The resulting mixture was stirred at 50 °C for 48 h, then diluted with 2 mL of MQ water and acidified with 1 M HCl until pH = 1. The mixture was extracted twice with 4 mL of CH_2Cl_2, washed with 2 mL of brine, dried over $MgSO_4$ and filtered. Upon elimination of the organic solvent, colourless oily mixtures of FAs were isolated.

For ^{13}C-NMR spectra recording, 100 mg of each FA mixture sample were dissolved in 1.4 mL of deuterated chloroform ($CDCl_3$) and 0.5 mL of this solution was placed in NMR tubes. Spectra were recorded on a Bruker AM-400 MHz spectrometer, operating at 100.6 MHz, at 305 K. Chemical shifts (δ) are expressed in ppm from the central signal of $CDCl_3$ (77.0 ppm). Before Fourier transformation, exponential multiplication with a LB = −0.7 was applied. Spectra were acquired using an inverse gated decoupling sequence so that fully decoupled spectra with no NOE were recorded. A total of 128 scans with a delay between scans of 60 s (>8 times T1) was used. Our analysis was centred on the methylene carbon resonances around 20–35 ppm [23].

For the integration, well-resolved signals of each acid were used. This gave a molar% average which was transformed into mass% average. However, the presence of palmitic and myristic acids in SOLF samples was observed by the splitting of the signals at 29.355 and 31.927 ppm. Unfortunately, this splitting does not give completely resolved signals. Therefore, the weight of these two acids was assigned higher error, although their combined contribution has the same general error of 5%.

2.3. Hematoxylin/Eosin Staining and Quantification of Adipocyte Size

WAT samples were fixed in 4% formaldehyde for 10 days, then washed with water (2 h), dehydrated with ethanol and subsequently embedded in paraffin. Thin serial sections (5 μm) were obtained with a vertical rotary microtome (Leica RM 2125RT) and mounted in glass-slides. Slices were stained with hematoxylin/eosin (H/E; Thermo Scientific, Madrid, Spain) to assess cellular morphology and diameter quantification. Two vision fields per slice were randomly selected and quantified. After staining, sections were dehydrated in ethanol and xylene and mounted with DPX. Samples were directly observed (20×) by using an Eclipse 50i-Nikon microscope equipped with a camera (DS-5M) and NIS-Elements software. Adipocyte-size classification was made according to the criteria provided by Verboven et al., 2018 [24].

2.4. Leptin Resistance Assessment in White Adipose Tissue

To evaluate whether leptin signalling in WAT was modified by the dietary treatment, an experiment aimed at evaluating the effect of exogenous leptin on LepR responsiveness was carried out after 8 weeks of dietary treatment. Mice received (i.p.) either saline or 1 mg/kg mouse recombinant leptin (Sigma, St. Louis, MO, USA) at 0900 h. After 60 min, animals were decapitated under isoflurane anaesthesia, blood was collected in chilled EDTA-coated polypropylene tubes, and Sc and Vis-WAT were dissected and stored at −80 °C until assay. Both tissues were prepared for Western blot, as described below. The dose of leptin was chosen based on previous studies of our group [11]. This dose has been shown to provide plasma leptin levels of approx. 80 ng/mL [11], which fits into the range of severe hyperleptinemia.

2.5. Western Blotting

For Western blotting, tissues were homogenized in ice-cold buffer containing 30 mM HEPES (pH = 7.9), 0.1 mM $Na_4P_2O_7$, 5 mM EDTA, 1% triton X-100, 0.5% glycerol, 1 μg/mL

aprotinin, 1 μg/mL leupeptin, 1 mM sodium fluoride, 7.5 mM trisodium orthovanadate. Tubes containing homogenates were frozen at −80 °C and thawed at 37 C three consecutive times, then centrifuged for 10 min at 4 °C. Equivalent amounts of proteins (50 μg) present in the supernatant were loaded in Laemli buffer (50 mM Tris, pH = 6.8, 10% sodium dodecyl sulphate, 10% glycerol, 5% mercaptoethanol, and 2 mg/mL blue bromophenol) and size-separated in 7% SDS-PAGE. Proteins were transferred to nitrocellulose membranes (GE Healthcare, Little Chalfont, Buckinghamshire, UK) using a transblot apparatus (Bio-Rad, Hercules, CA, USA). For immunoblotting, membranes were blocked with 5% non-fat dried milk in Tween-PBS for 1 h. Primary antibodies (Table 1) were applied at the convenient dilution, overnight at 4 °C. After washing, appropriate secondary antibodies (anti-rabbit, IgG-peroxidase conjugated) were applied for 1 h at a dilution of 1:2000.

Table 1. Primary antibodies used in Western blot.

Antigen	Manufacturer	Ref.	Host and Molecular Weight	Dilution
STAT3	Santa Cruz Biotechnology	Sc-483	Rabbit polyclonal, 86 kDa	1/250
pSTAT3 (Y705)	Cell Signalling	#9131	Rabbit polyclonal, 86 kDa	1/250
Akt	Cell Signalling	#9272	Rabbit polyclonal, 60 kDa	1/500
pAkt (S473)	Cell Signalling	#9271	Rabbit polyclonal, 60 kDa	1/500
AMPK	Cell Signalling	#2532	Rabbit polyclonal, 62 kDa	1/500
pAMPK (T172)	Cell Signalling	#2531	Rabbit polyclonal, 62 kDa	1/500
Anti-rabbit IgG	Santa Cruz Biotechnology	Sc-2357	Mouse monoclonal, HRP	1/2000

Blots were washed, incubated in enhanced chemiluminescence reagent (ECL Prime; GE Healthcare, Chicago, IL, USA), and bands were detected using the ChemiDoc XRS+ Imaging System (BioRad, Hercules, CA, USA). To ensure equal loading of samples, blots were re-incubated with β-actin monoclonal antibody (Affinity Bioreagents, Ancaster, CO, USA). Blots were detected using the ChemiDoc XRS+ Imaging System (Bio-Rad, Hercules, CA, USA). Values for pSTAT3, pAkt and pAMPK were normalized with STAT3, Akt and AMPK, respectively.

2.6. RNA Preparation and Quantitative Real-Time PCR

Total RNA from Sc and Vis-WAT was extracted by using the Tri-Reagent protocol (Sigma, St. Louis, MO, USA). cDNA was then synthesized from 1 μg total mRNA by using a high-capacity cDNA RT kit (Bio-Rad, Hercules, CA, USA). Quantitative RT-PCR was performed by using designed primer pairs (Integrated DNA Technologies, Coralville, IA, USA. Table 2). SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) was used for amplification according to the manufacturer's protocols, in CFX96 Real Time System (Bio-Rad, Hercules, CA, USA). Values were normalized to the housekeeping gene *Actb* and *18s*. The ΔΔC(T) method was used to determine relative expression levels. Statistics were performed using ΔΔC(T) values [25]. All samples were run in duplicate.

Table 2. Designed primer pairs used in this study.

mRNA	Forward (5′→3′)	Reverse (5′→3′)
Cebpa	CCGATGAGCAGTCACCTCC	AGGAACTCGTCGTTGAAGGC
Col1a1	ACTGCCCTCCTGACGCAT	AGAAAGCACAGCACTCGCC
Col3a1	CCCATGACTGTCCCACGTAAGCAC	TGGCCTGATCCATATAGGCAA-TACTG
Il1b	TGCCACCTTTTGACAGTGATG	TGATACTGCCTGCCTGAAGC
Il6	TACCACTTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTC

Pparg	CATGGTTGACACAGA-GATGCCATTCTG	TTGATCGCACTTTGGTATTCTT-GGAGC
Tnfa	GGTGCCTATGTCTCAGCCTC	GCTCCTCCACTTGGTGGTTT
Lepr	GCAGCAAAAGGAAGCATTGGA	GGTGAGGAGCAAGAGACTGG
18s	GGGAGCCTGAGAAACGGC	GGGTCGGGAGTGGGTAATTT
Actb	TGGTGG-GAATGGGTCAGAAGGACTC	CATGGCTGGGGTGTT-GAAGGTCTCA

2.7. Statistics

Adipocyte size distribution and diet-effect over leptin signalling were analysed by two-way ANOVA with a post-hoc Bonferroni correction. Individual effects were analysed by one-way ANOVA followed by a Bonferroni post-hoc analysis. Data were expressed as mean ± S.E.M. and statistical significance was set at $p < 0.05$. Normal distribution and variance homogeneity were assessed by means of the Bartlett and Brown–Forsyte test. Outliers were identified using the ROUT method (Q = 1%). All statistics were performed using GraphPad Prism software (GraphPad Software Inc. San Diego, CA, USA; Version 7.0a).

3. Results

3.1. UOLF, but Not SOLF, Preserved the Effect of Exogenous Leptin on STAT3, Akt and AMPK Phosphorylation Both in Subcutaneous and Visceral WAT

The effect of dietary treatment and acute leptin administration (1 mg/kg) on ^{705}Tyr-STAT3, ^{473}Ser-Akt and ^{172}Tyr-AMPK phosphorylation levels was evaluated both in Sc and Vis-WAT.

Subcutaneous WAT: As illustrated in Figure 1A–C, two-way ANOVA (see F values in Table 3) revealed an effect of leptin on relative pSTAT3 ($p < 0.001$), pAkt ($p < 0.001$), and pAMPK levels ($p < 0.001$), that was dependent on the type of diet consumed ($p < 0.05$, $p < 0.05$ and $p < 0.01$, respectively). Post-hoc analyses indicated that leptin increased pSTAT3, pAkt and pAMPK in control and UOLF mice but not in SOLF animals (see complete post-hoc analyses in Supplementary Table S2).

Visceral WAT: In this tissue, a significant effect of leptin on pSTAT3 (see F values in Table 4) ($p < 0.001$), pAkt ($p < 0.001$) and pAMPK relative levels ($p < 0.001$) was also detected. The effect of leptin depended on the type of diet consumed in the case of pSTAT3 ($p < 0.05$) and pAkt ($p < 0.01$) but not for pAMPK. Further post-hoc analysis revealed an effect of leptin on pSTAT3 and pAkt in SD and UOLF animals (see complete post-hoc analyses in Supplementary Table S3).

Table 3. F values obtained from two-way ANOVA to determine the effect of leptin and dietary treatment on ^{705}Tyr-STAT3, ^{473}Ser-AkT and ^{172}Tyr-AMPK phosphorylation in Sc-WAT.

	F Value		
Protein	**Leptin Effect**	**Diet Effect**	**Interaction Leptin x Diet**
pSTAT3/STAT3	$F_{(1,30)} = 42.89$; $p < 0.001$	$F_{(2,30)} = 6.890$; $p < 0.01$	$F_{(2,30)} = 4.393$; $p < 0.05$
pAkt/Akt	$F_{(1,30)} = 18.92$; $p < 0.001$	$F_{(2,30)} = 2.785$; $p = 0.07$	$F_{(2,30)} = 4.214$; $p < 0.05$
pAMPK/AMPK	$F_{(1,30)} = 17.86$; $p < 0.001$	$F_{(2,30)} = 1.321$; n.s.	$F_{(2,30)} = 5.466$; $p < 0.01$

Table 4. F values obtained from two-way ANOVA to determine the effect of leptin and dietary treatment on^{705}Tyr-STAT3, ^{473}Ser-pAkt and ^{172}Tyr-AMPK phosphorylation in Vis-WAT.

	F Value		
Protein	**Leptin Effect**	**Diet Effect**	**Interaction Leptin x Diet**
pSTAT3/STAT3	$F_{(1,30)} = 32.93$; $p < 0.001$	$F_{(2,30)} = 1.558$; n.s.	$F_{(2,30)} = 4.114$; $p < 0.05$
pAkt/Akt	$F_{(1,30)} = 27.75$; $p < 0.001$	$F_{(2,30)} = 4.705$; $p < 0.05$	$F_{(2,30)} = 5.727$; $p < 0.01$
pAMPK/AMPK	$F_{(1,30)} = 22.64$; $p < 0.001$	$F_{(2,30)} = 4.996$; $p < 0.05$	$F_{(2,30)} = 1.914$; n.s.

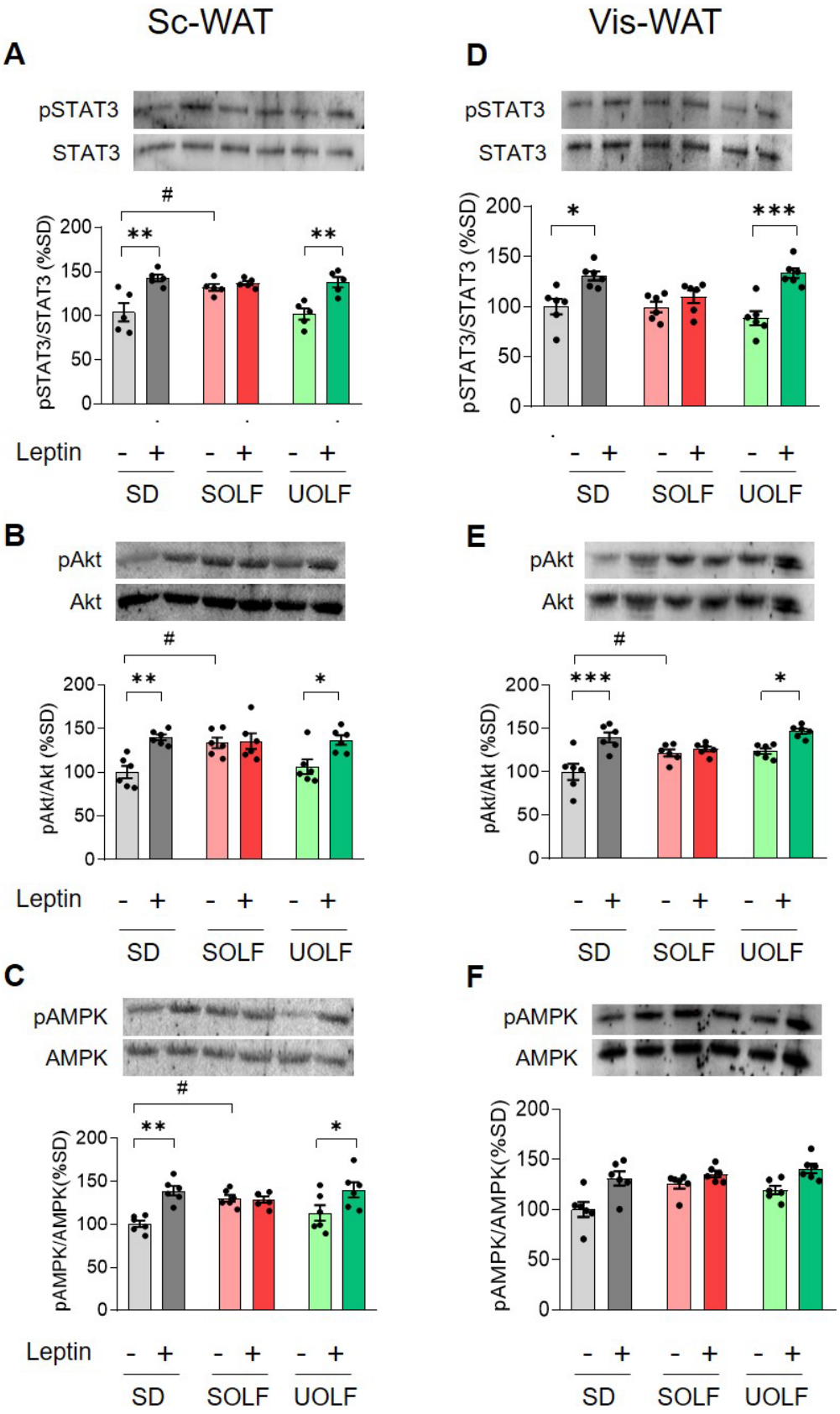


Figure 1. Influence of dietary treatment and acute leptin administration on phosphorylation levels of Tyr[705]-STAT3, Ser[473]-Akt and Tyr[172]-AMPK. Graphs show representative blots and relative levels of phosphorylated STAT3, Akt and AMPK in Sc-WAT (**A**–**C**) and Vis-WAT (**D**–**F**). Values are means

± S.E.M. ($n = 6$ per group). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.01$, compared to the corresponding saline group; # $p < 0.05$ compared to the saline-SD group (Bonferroni test).

3.2. SOLF and UOLF Increased Adipocyte Size Both in Subcutaneous and Visceral WAT

To evaluate the influence of dietary treatment with UOLF and SOLF on adipocyte size and number, Sc and Vis-WAT sections were stained with H/E. As illustrated in Figures 2A,C, H/E staining revealed that the average size of Sc adipocytes was larger in UOLF samples than in their corresponding controls (one-way ANOVA; $F_{(2,19)} = 4.02$, $p < 0.05$), while no effect of SOLF was detected. In the case of Vis-WAT (Figures 2B,D), the adipocyte size appeared also to be significantly greater in UOLF-treated mice than in control and SOLF-treated mice (one-way ANOVA; $F_{(2,19)} = 26.51$, $p < 0.001$), although SOLF adipocytes were also hypertrophic ($p < 0.05$).

Adipocyte-size distribution curves (Figures 2E,F) were also analysed. Two-way ANOVA revealed a significant influence of dietary treatment on adipocyte size distribution both in Sc-WAT (Figure 2E; $F_{(10,246)} = 3.243$, $p < 0.001$) and Vis-WAT (Figure 2F; $F_{(10,246)} = 19.64$, $p < 0.001$). Further one-way ANOVA indicated that the dietary treatment reduced the proportion of small adipocytes (0–1000 μm^2; Figure 1G) in Sc-WAT ($F_{(2,19)} = 3.497$, $p < 0.05$) and enhanced that of medium (1000–4000 μm^2; $F_{(2,19)} = 2.799$; $p = 0.07$) and large adipocytes (>4000 μm^2; $F_{(2,19)} = 2.690$; $p = 0.09$). In the case of Vis-WAT, small adipocytes (Figure 2H) were also reduced by the diets ($F_{(2,19)} = 34.18$, $p < 0.001$), while medium ($F_{(2,19)} = 15.33$, $p < 0.001$) and large adipocytes ($F_{(2,19)} = 19.98$, $p < 0.001$) were more abundant in these groups. It must be highlighted that in Vis-WAT, adipocyte-size distribution was different between SOLF and UOLF with small adipocytes being more abundant in SOLF samples ($p < 0.05$), and large adipocytes being more abundant in UOLF ($p < 0.01$).

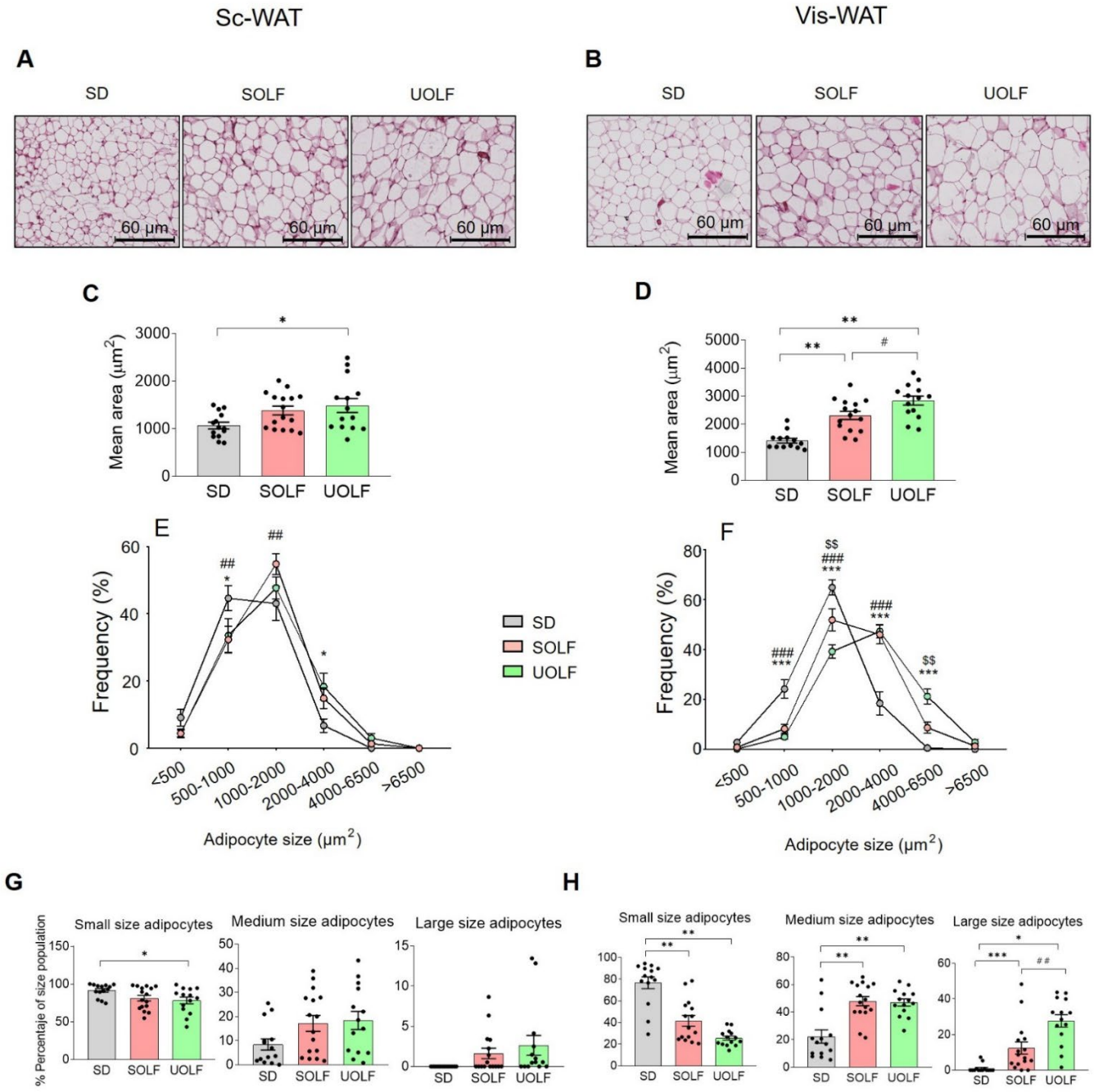


Figure 2. Effect of UOLF and SOLF diet on adipocyte morphology. Representative photomicrographs (×20) of H/E stained Sc (**A**) and Vis-WAT (**B**) from mice that consumed either SD, UOLF or SOLF. (**C**,**D**) graph bars show adipocyte mean areas in Sc and Vis-WAT, respectively. Graphs (**E**) and (**F**) illustrate adipocyte size distribution of Sc and Vis-WAT, respectively. Graphs (**G**,**H**) show the percentage of small, medium, and large adipocytes in Sc and Vis-WAT, respectively. Values are means ± S.E.M. (n = 6–7 per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to SD. $^{\#}$ $p < 0.05$ and $^{\#\#}$ $p < 0.01$ compared to SOLF (Bonferroni test).

3.3. UOLF and SOLF Inhibited the Expression of Genes Involved in White Adipocyte Differentiation but Neither the Expression of Pro-Inflammatory and Pro-Fibrotic Factors nor Leptin Receptor Genes Were Affected

The expression of peroxisome proliferator-activated receptor gamma (*Pparg*) and CCAAT enhancer binding protein α (*Cebpa*) genes was analysed both in Sc and Vis-WAT. In the Sc-WAT, UOLF and SOLF treatment failed to modulate *Cebpa* and *Pparg* gene

expression (Figure 3A). In contrast, in the Vis-WAT (Figure 3B) both *Cebpa* ($F_{(2,12)} = 52.06$, $p < 0.001$) and *Pparg* ($F_{(2,19)} = 12.96$, $p < 0.001$) expression was repressed by SOLF and UOLF.

Both diets were devoid of effect on the expression of the inflammation-related genes *Il1*, *Il6* and *Tnfa* both in Sc (Figure 3C) and Vis-WAT (Figure 3D), although the expression levels of *Tnfa* were at the limit of statistical significance. Similarly, SOLF and UOLF were without effect on gene expression of the pro-fibrotic gene *Col1a1*. An effect of SOLF was identified in the case of *Col3a1* in the Sc-WAT ($F_{(2,16)} = 6.483$, $p < 0.01$) (Figure 3 E,G).

The expression of the leptin receptor (*Lepr*) remained unchanged both in Sc and Vis-WAT (Figure 3F,H).

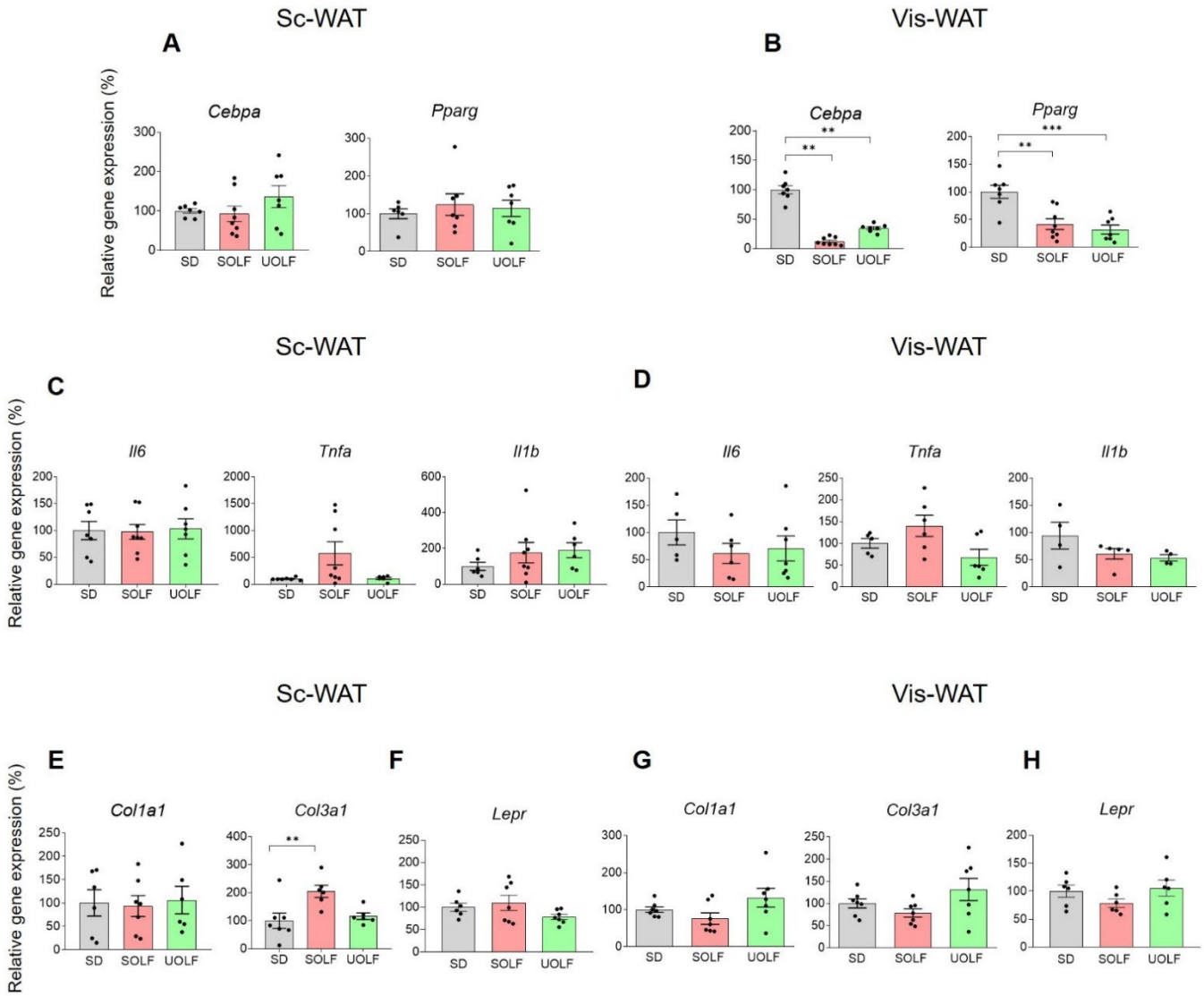


Figure 3. Effect of UOLF and SOLF on expression levels of the adipogenic genes *Pparg* and *Cebpa* in Sc and Vis-WAT. Figure (**A**) shows the lack of effect of SOLF and UOLF on mRNA levels of *Cebpa* and *Pparg* in Sc-WAT. In contrast, in Vis-WAT, both SOLF and UOLF down-regulated the expression of these genes (**B**). Figures (**C**,**D**) show mRNA levels of *Il1*, *Il6* and *Tnfa* in Sc and Vis-WAT, respectively. Figures (**E**,**G**) show the expression levels of *Col1a1* and *Col3a1* in Sc and Vis-WAT, respectively. Figures (**F**,**H**) show the lack of effect of SOLF and UOLF on expression levels of *Lepr* in Sc and Vis-WAT, respectively. Values are means ± S.E.M. ($n = 6$–7 per group). ** $p < 0.01$, *** $p < 0.001$, compared to their respective controls (Bonferroni test).

3.4. SOLF and UOLF Differently Affect the Composition of Subcutaneous and Visceral WAT

As appears summarized in Table 5, SOLF and UOLF treatment differently affected the composition of both Vis and Sc-WAT. In the case of Vis-WAT, UOLF treatment triggered a 40% increase of oleic acid content to the detriment of palmitic acid. In contrast, SOLF treatment barely modified the proportion of oleic acid but promoted the accumulation of both myristic (25%) and lauric (22%) acids, which were absent in control and UOLF Vis-WAT.

With regard to Sc-WAT composition, UOLF treatment also triggered a dramatic increase in oleic acid content (46% increment) to the detriment of palmitic acid (23.5% decrease) and linoleic acid (22% decrease). Treatment with SOLF led to a small decrease in both palmitic (10%) and linoleic acid content (17%), and promoted the storage of myristic and lauric acids, which were also absent in control and UOLF tissues.

Table 5. Fatty acid composition of Vis- and Sc-WAT.

	Vis-WAT					
	Standard Diet		**SOLF**		**UOLF**	
FA	**Mol%**	**Mass%**	**Mol%**	**Mass%**	**Mol%**	**Mass%**
Oleic	45.4 ± 2.3	47.9 ± 2.4	36.5 ± 1.8	42.3 ± 2.1	87.7 ± 4.4	89.0 ± 4.4
Palmitic	54.5 ± 2.7	52.1 ± 2.6	10.2 ± 0.5	10.8 ± 0.5	12.2 ± 0.6	11.0 ± 0.5
Myristic	ND	ND	26.6 ± 1.3	25.0 ± 1.2	ND	ND
Lauric	ND	ND	26.6 ± 1.3	21.9 ± 1.1	ND	ND
Linoleic	ND	ND	ND	ND	ND	ND
	Sc-WAT					
Oleic	35.6 ± 1.8	36.8 ± 1.8	37.7 ± 1.9	41.6 ± 2.1	81.9 ± 4.1	82.7 ± 4.1
Palmitic	33.1 ± 1.6	31.1 ± 1.5	20.0 ± 1.0	20.0 ± 1.0	8.1 ± 0.4	7.5 ± 0.4
Myristic	ND	ND	9.8 ± 0.5	8.7 ± 0.4	ND	ND
Lauric	ND	ND	18.5 ± 0.9	14.4 ± 0.7	ND	ND
Linoleic	31.3 ± 1.6	32.1 ± 1.6	13.9 ± 0.7	15.5 ± 0.8	9.8 ± 0.5	9.8 ± 0.5

Data are means ± S.E.M. of 3–4 samples/group. ND, not detected.

4. Discussion

Leptin drives the crosstalk between WAT and organs/tissues regulating energy balance and is involved in the endocrine and autoparacrine control of energy metabolism [26–28]. In the current study, we have shown that long-term consumption of diets enriched in saturated FA (SOLF) affects the phosphorylation levels of protein kinases integral to LepR signalling pathways and LepR responsiveness to leptin in WAT. In contrast, unsaturated diets (UOLF) were devoid of these effects. Nevertheless, both SOLF and UOLF had a significant impact on WAT remodelling.

One of the most remarkable set of data in our study is the increase in basal pSTAT3, pAkt and pAMPK observed specifically in SOLF mice, particularly in the Sc-WAT. This is an interesting point since we previously reported that WAT leptin expressions as well as plasma leptin levels are higher in UOLF than in SOLF mice [12,29] and, therefore, one would expect the activation of these signalling kinases in UOLF rather than in SOLF animals. We speculate that this effect may be integral to an adaptive response aimed at increasing adipogenesis and/or lipogenesis in Sc-WAT, since STAT3, Akt and AMPK have been shown to regulate a substantial number of substrates involved in these processes [8,30,31]. Notably, STAT3 is abundantly expressed in pre-adipocytes [32] and pSTAT3 has been shown to contribute, at least in vitro, to adipocyte differentiation [33]. The role of Akt has been better established as it is involved both in the lipogenic and adipogenic effects of insulin [34]. With regard to AMPK, the increase in phosphorylated ^{172}Tyr-AMPK elicited by SOLF is coherent with the inhibition of lipogenic pathways triggered by HFDs and also with the prominent role of AMPK on adipocyte proliferation [35,36]. This finding

suggests that the endocrine environment generated by SOLF has consequences for signalling pathways relevant for Sc-WAT adaptation to the metabolic challenge evoked by an excess of saturated fat. In this context, the apparent lack of effect of leptin on STAT3/Akt/AMPK phosphorylation observed in SOLF mice is of difficult interpretation since leptin effects could be masked by the above-mentioned diet effect. In contrast, in the Vis-WAT, SOLF only increased basal phosphorylation levels of Akt, although a desensitization of LepR to leptin effects was globally observed in this tissue. With respect to UOLF effects, it is noteworthy that the phosphorylation levels of STAT3, Akt and AMPK were not affected by the diet itself although they were enhanced by leptin in a similar way that leptin did in animals consuming regular chow. This circumstance was observed both in Sc and Vis-WAT, demonstrating that unsaturated diets fully preserve LepR responsiveness in WAT and indicating that the autoparacrine effect of leptin is not affected by these diets. In any case, differences in LepR sensitivity between dietary treatments are not linked to different levels of LepR density since no change was detected in the expression of the *Lepr*.

Altogether, the results regarding kinase phosphorylation show that SOLF has a greater impact than UOLF on the autoparacrine effect of leptin. On the basis of previous studies, showing that the rise of leptin levels is more prominent in UOLF than in SOLF-treated mice [12,29], our current findings suggest the involvement of other factors than leptin on STAT3/Akt/AMPK signalling dysregulation. In this line, inflammation evoked by saturated FA could contribute to SOLF effects [37]. Nevertheless, although our data concerning the impact of SOLF/UOLF on WAT inflammation are restricted to the quantification of *Il6*, *Il1* and *Tnfa* mRNAs, they suggest that local inflammatory mediators do not play a major role in SOLF effects. In the same way, fibrosis does not appear to be a prominent hallmark of SOLF and UOLF WAT since the expression of the fibrosis markers *Col1a1* and *Col3a1* remained unchanged in Vis-WAT and only an increase in *Col3a1* was detected in the Sc-WAT of SOLF mice. Such an increase may be eventually related to actin re-organization required for adipogenesis [38]. Finally, some studies point to a negative effect of excessive adipocyte FA uptake on LepR functionality [39]; related to that, palmitic acid has been shown to impair LepR signalling within the hypothalamus [40], a finding that would point to a particular effect of saturated FA on LepR signalling. It is noteworthy that both SOLF and UOLF contain palmitic acid, while lauric acid is only present in SOLF. Although lauric acid is currently considered as a FA with less impact than palmitic acid on WAT inflammation [41], our results indicate that lauric acid does not prevent the harmful consequences of saturated fat intake. Related to that, our findings suggest that human dietary habits including foods enriched in lauric acid (i.e., coconut oil) should be analysed with a view to properly evaluating their eventual health risks.

Taken together with the morphological data, the effect of SOLF/UOLF on LepR signalling points to an adaptive remodelling in Sc-WAT. In fact, although adipocyte size distribution was similar in SOLF, UOLF and control Sc-WAT, both SOLF and UOLF mice displayed larger fat depots than control animals [12], allowing us to conclude that the adipocyte number in Sc-WAT is larger in SOLF/UOLF than in control mice. This supports the existence of active adipogenesis, a circumstance that is coherent with the basal increase of phosphorylated kinases identified in SOLF mice as well as in UOLF-treated mice receiving an acute leptin challenge.

In contrast, both diets decreased the proportion of small adipocytes and enhanced that of large adipocytes in Vis-WAT, leading us to hypothesize that SOLF/UOLF similarly limit adipogenesis in this tissue. This hypothesis is corroborated by the down-regulation in the expression of the adipogenic genes, *Cebpa* and *Pparg* [42], which remained unmodified in Sc-WAT. Collectively, our data indicate that both SOLF and UOLF promote adipocyte hypertrophy but inhibit adipogenesis in Vis-WAT. Despite the lack of sufficient data regarding the presence of inflammation and/or fibrosis, we speculate that 8-week SOLF/UOLF intake leads to an incipient pathologic remodelling of Vis-WAT [43]. In any case, further studies aimed at characterizing the expression of inflammatory markers in

the isolated stromal fraction of WAT would be necessary for a proper characterization of this issue. Similar considerations can be made regarding fibrosis [39].

Otherwise, we have identified significant differences between SOLF, UOLF and control mice regarding WAT composition. We want to highlight that WAT composition was analysed by means of ^{13}C-NMR, which is less performant than gas chromatography for this kind of study. In any case, ^{13}C-NMR is also considered a reliable method to analyse lipids in complex mixtures [44]. Thus, compared to control Vis-WAT [unsaturated FA]/[saturated FA], ratios were moderately decreased in SOLF (0.6 vs. 0.8) and enhanced in UOLF animals (7.2 vs. 0.8). This pattern, which was also observed in the Sc-WAT (1.1 vs. 2.0, SOLF vs. controls; 11.4 vs. 2.0, UOLF vs. controls), is coherent with diet compositions and introduces an interesting point of discussion, related to the influence that the mechanical properties of WAT may have on WAT functionality [45]. Thus, despite the lack of proper biophysical data, one could hypothesize that UOLF WAT would be more fluid than SOLF and control WATs. In fact, melting points of TGs containing oleyl and linoleyl moieties (which presumably are major components of UOLF Vis and Sc-WAT), would be around 5 °C, and therefore liquid at mouse body temperature, while TGs contained in control and SOLF WAT should be solid at this temperature. Reasoning in this way, SOLF WAT TGs should present higher melting points than control WAT TGs. With all that in mind, variations in WAT rigidity may account for some of the differences detected between SOLF and UOLF, regarding size adipocyte distribution and LepR signalling. In fact, adipogenesis is a mechanosensitive process, and increasing evidence links lipid production in adipocytes to a particular mechanical environment [46]. This circumstance, together with the differential effect of saturated and unsaturated FA on adipocyte maturation [47], allows us to speculate that differences between SOLF and UOLF mice regarding *Lep* gene expression previously reported by us [12,29] may be linked to the biophysical properties of the tissue [45,48,49].

Similar considerations could be made to interpret differences in LepR signalling. Interestingly, membrane protein conformation and activity have been shown to depend on the membrane lipid environment [50], which may be theoretically modified by SOLF/UOLF [51]. Specifically, membrane cell composition and fluidity have been shown to have a direct impact on membrane receptor functionality [52,53].

Another interesting point of our study deals with the reduction in linoleic acid content evoked both by SOLF and UOLF in the Sc-WAT. Since Ω-6 PUFA have been shown to promote inflammation [54], reduction in linoleic acid content by SOLF/UOLF may balance other detrimental effects elicited by these diets, and explain why Sc-WAT is less sensitive to SOLF/UOLF effects than Vis-WAT. In any case, further experiments need to be carried out to confirm the differential effects of saturated vs. unsaturated fats on mechanical properties/stiffness and fluidity of white adipocyte membranes.

It has to be noted that our study was carried out only on male mice and, therefore, complementary studies carried out on females are necessary to further characterize sex-dependent differences. With regard to the eventual translation of our results to humans, the finding that SOLF triggers a similar increase in adiposity to UOLF but causes a deficit in leptin production and impairs LepR signalling supports the concept that an excess of lauric acid (i.e., coconut oil) may be detrimental for leptin effects and therefore for WAT function.

In summary, despite both SOLF and UOLF having a similar effect on WAT expansion, these diets have a different impact on LepR signalling within Sc and Vis-WAT, with SOLF-treated mice displaying apparent leptin resistance, particularly in the Vis-WAT. Our findings show that the intake of food enriched with either oleic (UOLF) or lauric/palmitic acids (SOLF) may differently affect the autoparacrine role of leptin. Overall, our findings suggest that SOLF desensitize signalling pathways downstream of LepR in adipocytes, which could limit WAT adaptive mechanisms. Nevertheless, and despite the fact that UOLF does not alter LepR signalling, this diet triggers adipocyte hypertrophy to a similar extent to SOLF, indicating that, regardless of diet composition, an excessive intake

of fat is harmful to WAT function. Further research aimed at correlating LepR responsiveness with adipocyte function in SOLF- and UOLF-fed mice appears necessary.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomedicines10081864/s1, Table S1: Diet composition, Table S2. Bonferroni adjusted P values corresponding to the effect of dietary treatment and acute leptin administration on relative phosphorylation levels of Tyr^{705}-STAT3, Ser^{473}-Akt and Tyr^{172}-AMPK in Sc-WAT, Table S3. Bonferroni adjusted P values corresponding to the effect of dietary treatment and acute leptin administration on relative phosphorylation levels of Tyr^{705}-STAT3, Ser^{473}-Akt and Tyr^{172}-AMPK in Vis-WAT.

Author Contributions: J.F.-F., A.P., G.D., J.P.-C. and F.C. contributed to data acquisition and interpretation. A.P., M.R.-G. and B.M. designed the study and wrote the manuscript. J.P.-C., V.C., N.D.O. and M.R.-G. drafted and revised the MS. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Ministerio de Ciencia e Innovación (MCI) (BFU2016-78556-R, PID2020-117422RB-C21), the European Regional Development Fund and the Fundación Universitaria San Pablo-CEU (FUSP-CEU), Spain. A.P. and J.F.-F. were supported by grants from MCI (BES-2013-063773) and FUSP-CEU, respectively. F.C. was supported by the Erasmus Program (Università della Sapienza, Rome, Italy).

Institutional Review Board Statement: The study was conducted according to the guidelines of the European Union Laboratory Animal Care Rules (86/609/EEC) and was approved by the Animal Research Committee of San Pablo CEU University (PCD Ref. PROEX-017/18. Approval date. 23 March 2017).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: We thank J.M. Garrido, J. Bravo and I. Bordallo for skilful animal care during the experiment.

Conflicts of Interest: The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this reported research.

References

1. Grundy, S.M. Adipose tissue and metabolic syndrome: Too much, too little or neither. *Eur. J. Clin. Investig.* **2015**, *45*, 1209–1217. https://doi.org/10.1111/eci.12519.
2. Wronska, A.; Kmiec, Z. Structural and biochemical characteristics of various white adipose tissue depots. *Acta Physiol.* **2012**, *205*, 194–208. https://doi.org/10.1111/j.1748-1716.2012.02409.x.
3. Romacho, T.; Elsen, M.; Röhrborn, D.; Eckel, J. Adipose tissue and its role in organ crosstalk. *Acta Physiol.* **2014**, *210*, 733–753. https://doi.org/10.1111/apha.12246.
4. Trayhurn, P.; Wood, I.S. Adipokines: Inflammation and the pleiotropic role of white adipose tissue. *Br. J. Nutr.* **2004**, *92*, 347–355. https://doi.org/10.1079/bjn20041213.
5. Zhu, Q.; Glazier, B.J.; Hinkel, B.C.; Cao, J.; Liu, L.; Liang, C.; Shi, H. Neuroendocrine Regulation of Energy Metabolism Involving Different Types of Adipose Tissues. *Int. J. Mol. Sci.* **2019**, *20*, 2707. https://doi.org/10.3390/ijms20112707.
6. Martínez-Sánchez, N. There and Back Again: Leptin Actions in White Adipose Tissue. *Int. J. Mol. Sci.* **2020**, *21*, 6039. https://doi.org/10.3390/ijms21176039.
7. Considine, R.V.; Sinha, M.K.; Heiman, M.L.; Kriauciunas, A.; Stephens, T.W.; Nyce, M.R.; Ohannesian, J.P.; Marco, C.C.; McKee, L.J.; Bauer, T.L.; et al. Serum Immunoreactive-Leptin Concentrations in Normal-Weight and Obese Humans. *N. Engl. J. Med.* **1996**, *334*, 292–295. https://doi.org/10.1056/NEJM199602013340503.
8. Machinal-Quélin, F.; Dieudonné, M.N.; Leneveu, M.C.; Pecquery, R.; Giudicelli, Y. Proadipogenic effect of leptin on rat preadipocytes in vitro: Activation of MAPK and STAT3 signaling pathways. *Am. J. Physiol. Physiol.* **2002**, *282*, C853–C863. https://doi.org/10.1152/ajpcell.00331.2001.
9. Rhee, S.D.; Sung, Y.-Y.; Jung, W.H.; Cheon, H.G. Leptin inhibits rosiglitazone-induced adipogenesis in murine primary adipocytes. *Mol. Cell. Endocrinol.* **2008**, *294*, 61–69. https://doi.org/10.1016/j.mce.2008.08.018.
10. Guzmán-Ruiz, R.; Stucchi, P.; Ramos, M.P.; Sevillano, J.; Somoza, B.; Fernández-Alfonso, M.; Ruiz-Gayo, M. Leptin drives fat distribution during diet-induced obesity in mice. *Endocrinol. Nutr.* **2012**, *59*, 354–361. https://doi.org/10.1016/j.endonu.2012.04.001.

11. Guzmán-Ruiz, R.; Somoza, B.; Gil-Ortega, M.; Merino, B.; Cano, V.; Attané, C.; Castan-Laurell, I.; Valet, P.; Fernández-Alfonso, M.S.; Ruiz-Gayo, M. Sensitivity of Cardiac Carnitine Palmitoyltransferase to Malonyl-CoA Is Regulated by Leptin: Similarities with a Model of Endogenous Hyperleptinemia. *Endocrinology* **2010**, *151*, 1010–1018. https://doi.org/10.1210/en.2009-1170.
12. Plaza, A.; Antonazzi, M.; Blanco-Urgoiti, J.; Del Olmo, N.; Ruiz-Gayo, M. Potential Role of Leptin in Cardiac Steatosis Induced by Highly Saturated Fat Intake during Adolescence. *Mol. Nutr. Food Res.* **2019**, *63*, 1900110. https://doi.org/10.1002/mnfr.201900110.
13. Stucchi, P.; Guzmán-Ruiz, R.; Gil-Ortega, M.; Merino, B.; Somoza, B.; Cano, V.; de Castro, J.; Sevillano, J.; Ramos, M.P.; Fernández-Alfonso, M.S.; et al. Leptin resistance develops spontaneously in mice during adult life in a tissue-specific manner. Consequences for hepatic steatosis. *Biochimie* **2011**, *93*, 1779–1785. https://doi.org/10.1016/j.biochi.2011.06.020.
14. Mu¨nzberg, H.; Flier, J.S.; Bjørbæk, C. Region-Specific Leptin Resistance within the Hypothalamus of Diet-Induced Obese Mice. *Endocrinology* **2004**, *145*, 4880–4889. https://doi.org/10.1210/en.2004-0726.
15. Rahmouni, K.; Morgan, D.A.; Morgan, G.M.; Mark, A.L.; Haynes, W.G. Role of Selective Leptin Resistance in Diet-Induced Obesity Hypertension. *Diabetes* **2005**, *54*, 2012–2018. https://doi.org/10.2337/diabetes.54.7.2012.
16. Wang, M.-Y.; Orci, L.; Ravazzola, M.; Unger, R.H. Fat storage in adipocytes requires inactivation of leptin's paracrine activity: Implications for treatment of human obesity. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 18011–18016. https://doi.org/10.1073/pnas.0509001102.
17. Unger, R.H. Lipotoxic Diseases. *Annu. Rev. Med.* **2002**, *53*, 319–336. https://doi.org/10.1146/annurev.med.53.082901.104057.
18. Unger, R.H.; Zhou, Y.-T.; Orci, L. Regulation of fatty acid homeostasis in cells: Novel role of leptin. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 2327–2332. https://doi.org/10.1073/pnas.96.5.2327.
19. Wang, M.-Y.; Grayburn, P.; Chen, S.; Ravazzola, M.; Orci, L.; Unger, R.H. Adipogenic capacity and the susceptibility to type 2 diabetes and metabolic syndrome. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6139–6144. https://doi.org/10.1073/pnas.0801981105.
20. Couillard, C.; Mauriège, P.; Imbeault, P.; Prud'homme, D.; Nadeau, A.; Tremblay, A.; Bouchard, C.; Després, J.-P. Hyperleptinemia is more closely associated with adipose cell hypertrophy than with adipose tissue hyperplasia. *Int. J. Obes.* **2000**, *24*, 782–788. https://doi.org/10.1038/sj.ijo.0801227.
21. Lee, Y.; Yu, X.; Gonzales, F.; Mangelsdorf, D.J.; Wang, M.-Y.; Richardson, C.; Witters, L.A.; Unger, R.H. PPARα is necessary for the lipopenic action of hyperleptinemia on white adipose and liver tissue. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11848–11853. https://doi.org/10.1073/pnas.182420899.
22. Herrera, E.; Ayanz, A. Calculation of lipolysis and esterification from glycerol metabolism in rat adipose tissue. *J. Lipid Res.* **1972**, *13*, 802–809.
23. Giraudeau, P.; Baguet, E. Improvement of the inverse-gated-decoupling sequence for a faster quantitative analysis of various samples by 13C NMR spectroscopy. *J. Magn. Reson.* **2006**, *180*, 110–117. https://doi.org/10.1016/j.jmr.2006.01.015.
24. Verboven, K.; Wouters, K.; Gaens, K.; Hansen, D.; Bijnen, M.; Wetzels, S.; Stehouwer, C.D.; Goossens, G.; Schalkwijk, C.G.; Blaak, E.E.; et al. Abdominal subcutaneous and visceral adipocyte size, lipolysis and inflammation relate to insulin resistance in male obese humans. *Sci. Rep.* **2018**, *8*, 4677. https://doi.org/10.1038/s41598-018-22962-x.
25. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT Method. *Methods* **2001**, *25*, 402–408. https://doi.org/10.1006/meth.2001.1262.
26. Ahima, R.S.; Flier, J.S. Adipose Tissue as an Endocrine Organ. *Trends Endocrinol. Metab.* **2000**, *11*, 327–332. https://doi.org/10.1016/s1043-2760(00)00301-5.
27. Dunmore, S.J.; Brown, J.E.P. The role of adipokines in β-cell failure of type 2 diabetes. *J. Endocrinol.* **2012**, *216*, T37–T45. https://doi.org/10.1530/JOE-12-0278.
28. Stern, J.H.; Rutkowski, J.M.; Scherer, P.E. Adiponectin, Leptin, and Fatty Acids in the Maintenance of Metabolic Homeostasis through Adipose Tissue Crosstalk. *Cell Metab.* **2016**, *23*, 770–784. https://doi.org/10.1016/j.cmet.2016.04.011.
29. Fernández-Felipe, J.; Merino, B.; Sanz-Martos, A.; Plaza, A.; Contreras, A.; Naranjo, V.; Morales, L.; Chowen, J.; Cano, V.; Ruiz-Gayo, M.; et al. Saturated and unsaturated fat diets impair hippocampal glutamatergic transmission in adolescent mice. *Psychoneuroendocrinology* **2021**, *133*, 105429. https://doi.org/10.1016/j.psyneuen.2021.105429.
30. Zhang, K.; Guo, W.; Yang, Y.; Wu, J. JAK2/STAT3 pathway is involved in the early stage of adipogenesis through regulatingC/EBPβtranscription. *J. Cell. Biochem.* **2011**, *112*, 488–497. https://doi.org/10.1002/jcb.22936.
31. Harris, R.B. Direct and indirect effects of leptin on adipocyte metabolism. *Biochim. Biophys. Acta* **2013**, *1842*, 414–423. https://doi.org/10.1016/j.bbadis.2013.05.009.
32. Deng, J.; Hua, K.; Lesser, S.S.; Harp, J.B. Activation of Signal Transducer and Activator of Transcription-3 during Proliferative Phases of 3T3-L1 Adipogenesis. *Endocrinology* **2000**, *141*, 2370–2376. https://doi.org/10.1210/endo.141.7.7551.
33. Richard, A.J.; Stephens, J.M. The role of JAK–STAT signaling in adipose tissue function. *Biochim. Biophys. Acta* **2013**, *1842*, 431–439. https://doi.org/10.1016/j.bbadis.2013.05.030.
34. Taniguchi, C.M.; Emanuelli, B.; Kahn, C.R. Critical nodes in signalling pathways: Insights into insulin action. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 85–96. https://doi.org/10.1038/nrm1837.
35. Habinowski, S.A.; Witters, L.A. The Effects of AICAR on Adipocyte Differentiation of 3T3-L1 Cells. *Biochem. Biophys. Res. Commun.* **2001**, *286*, 852–856. https://doi.org/10.1006/bbrc.2001.5484.
36. Villena, J.A.; Viollet, B.; Andreelli, F.; Kahn, A.; Vaulont, S.; Sul, H.S. Induced Adiposity and Adipocyte Hypertrophy in Mice Lacking the AMP-Activated Protein Kinase-α2 Subunit. *Diabetes* **2004**, *53*, 2242–2249. https://doi.org/10.2337/diabetes.53.9.2242.

37. Fritsche, K.L. The Science of Fatty Acids and Inflammation. *Adv. Nutr. Int. Rev. J.* **2015**, *6*, 293S–301S. https://doi.org/10.3945/an.114.006940.
38. Al Hasan, M.; Martin, P.; Shu, X.; Patterson, S.; Bartholomew, C. Type III Collagen is Required for Adipogenesis and Actin Stress Fibre Formation in 3T3-L1 Preadipocytes. *Biomolecules* **2021**, *11*, 156. https://doi.org/10.3390/biom11020156.
39. Hajri, T.; Hall, A.M.; Jensen, D.R.; Pietka, T.A.; Drover, V.A.; Tao, H.; Eckel, R.; Abumrad, N.A. CD36-Facilitated Fatty Acid Uptake Inhibits Leptin Production and Signaling in Adipose Tissue. *Diabetes* **2007**, *56*, 1872–1880. https://doi.org/10.2337/db06-1699.
40. Cheng, L.; Yu, Y.; Szabo, A.; Wu, Y.; Wang, H.; Camer, D.; Huang, X.-F. Palmitic acid induces central leptin resistance and impairs hepatic glucose and lipid metabolism in male mice. *J. Nutr. Biochem.* **2015**, *26*, 541–548. https://doi.org/10.1016/j.jnutbio.2014.12.011.
41. Saraswathi, V.; Kumar, N.; Gopal, T.; Bhatt, S.; Ai, W.; Ma, C.; Talmon, G.; Desouza, C. Lauric Acid versus Palmitic Acid: Effects on Adipose Tissue Inflammation, Insulin Resistance, and Non-Alcoholic Fatty Liver Disease in Obesity. *Biology* **2020**, *9*, 346. https://doi.org/10.3390/biology9110346.
42. Farmer, S.R. Transcriptional control of adipocyte formation. *Cell Metab.* **2006**, *4*, 263–273. https://doi.org/10.1016/j.cmet.2006.07.001.
43. Vishvanath, L.; Gupta, R.K. Contribution of adipogenesis to healthy adipose tissue expansion in obesity. *J. Clin. Investig.* **2019**, *129*, 4022–4031. https://doi.org/10.1172/JCI129191.
44. Plaza, A.; Merino, B.; Cano, V.; Domínguez, G.; Pérez-Castells, J.; Fernandez-Alfonso, M.S.; Sengenes, C.; Chowen, J.A.; Ruiz-Gayo, M. Cholecystokinin is involved in triglyceride fatty acid uptake by rat adipose tissue. *J. Endocrinol.* **2018**, *236*, 137–150. https://doi.org/10.1530/JOE-17-0580.
45. Shoham, N.; Girshovitz, P.; Katzengold, R.; Shaked, N.T.; Benayahu, D.; Gefen, A. Adipocyte Stiffness Increases with Accumulation of Lipid Droplets. *Biophys. J.* **2014**, *106*, 1421–1431. https://doi.org/10.1016/j.bpj.2014.01.045.
46. Shoham, N.; Gefen, A. Mechanotransduction in adipocytes. *J. Biomech.* **2011**, *45*, 1–8. https://doi.org/10.1016/j.jbiomech.2011.10.023.
47. Hsieh, C.-M.; Liu, P.Y.; Chin, L.K.; Zhang, J.B.; Wang, K.; Sung, K.-B.; Ser, W.; Bourouina, T.; Leprince-Wang, Y.; Liu, A.-Q. Regulation of lipid droplets in live preadipocytes using optical diffraction tomography and Raman spectroscopy. *Opt. Express* **2019**, *27*, 22994–23008. https://doi.org/10.1364/OE.27.022994.
48. Hossain, G.; Iwata, T.; Mizusawa, N.; Shima, S.W.N.; Okutsu, T.; Ishimoto, K.; Yoshimoto, K. Compressive force inhibits adipogenesis through COX-2-mediated down-regulation of PPARγ2 and C/EBPα. *J. Biosci. Bioeng.* **2010**, *109*, 297–303. https://doi.org/10.1016/j.jbiosc.2009.09.003.
49. Maddalozzo, G.F.; Iwaniec, U.T.; Turner, R.T.; Rosen, C.J.; Widrick, J.J. Whole-body vibration slows the acquisition of fat in mature female rats. *Int. J. Obes.* **2008**, *32*, 1348–1354. https://doi.org/10.1038/ijo.2008.111.
50. Phillips, R.; Ursell, T.; Wiggins, P.; Sens, P. Emerging roles for lipids in shaping membrane-protein function. *Nature* **2009**, *459*, 379–385. https://doi.org/10.1038/nature08147.
51. Abbott, S.K.; Else, P.L.; Atkins, T.A.; Hulbert, A. Fatty acid composition of membrane bilayers: Importance of diet polyunsaturated fat balance. *Biochim. Biophys. Acta* **2012**, *1818*, 1309–1317. https://doi.org/10.1016/j.bbamem.2012.01.011.
52. Desai, A.J.; Miller, L.J. Changes in the plasma membrane in metabolic disease: Impact of the membrane environment on G protein-coupled receptor structure and function. *Br. J. Pharmacol.* **2017**, *175*, 4009–4025. https://doi.org/10.1111/bph.13943.
53. Ge, G.; Wu, J.; Lin, Q. Effect of Membrane Fluidity on Tyrosine Kinase Activity of Reconstituted Epidermal Growth Factor Receptor. *Biochem. Biophys. Res. Commun.* **2001**, *282*, 511–514. https://doi.org/10.1006/bbrc.2001.4600.
54. Sellem, F.; Pesando, D.; Bodennec, G.; Girard, J.-P.; Simopoulos, A.P. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. *Nutrients* **2016**, *8*, 128. https://doi.org/10.3390/nu8030128.

DISCUSIÓN

Numerosos investigadores han demostrado que la ingesta de dietas ricas en grasas (HFDs) deteriora los procesos de memoria y aprendizaje dependientes de hipocampo (**Dinel y cols., 2011; Hoane y cols., 2011; Valladolid-Acebes y cols., 2011**) así como los mecanismos responsables del control hipotalámico de la ingesta y el equilibrio energético (**Dickson y Chowen, 2020; Kim y Choi, 2013**), especialmente cuando se consumen durante la adolescencia. Así mismo, estas dietas inducen cambios en la función endocrina del tejido adiposo, que está mediada por hormonas como la leptina y la adiponectina.

La leptina actúa en áreas cerebrales como el hipotálamo, el hipocampo y la corteza prefrontal, donde regula funciones como la ingesta, la memoria y la cognición (**Preston y Eichenbaum, 2013; Timper y Brüning, 2017**), lo que sugiere la existencia de un eje tejido adiposo-SNC que podría verse alterado por el consumo excesivo de HFDs (**Shimizu y Mori, 2005**). Estas dietas presentan alto contenido en grasas saturadas, grasas insaturadas y azúcares, lo que dificulta la identificación del componente de la dieta responsable del daño en el SNC.

Los resultados de esta Tesis Doctoral muestran que el consumo de dietas ricas en grasas saturadas (SOLF) o monoinsaturadas (UOLF) induce alteraciones en el eje adiposo-SNC que dependen del tipo de grasa consumida y que podrían estar causadas por un desajuste hormonal, por un efecto directo de las grasas o por la interacción entre ambos.

Uno de los resultados más relevantes de nuestro estudio muestra que la ingesta de dieta SOLF durante 8 semanas aumenta la cantidad de tejido adiposo pero reprime el gen de leptina en el TAB-Vis y apenas modifica la concentración de leptina en plasma (**Fernández-Felipe y cols., 2021; Plaza y cols., 2019**). Este hecho resulta especialmente significativo ya que el aumento en el peso de los tejidos adiposos conlleva habitualmente un aumento proporcional de leptina plasmática (**Cammisotto y cols., 2003; Ahima y Osei, 2004**). De hecho, esta proporcionalidad sí se mantiene con la dieta UOLF. Teniendo en cuenta que la leptina es una hormona catabólica esencial en el

metabolismo energético, la normoleptinemia que presentan los animales SOLF, junto con el incremento de los NEFA plasmáticos en estado postprandial (**Fernández-Felipe y cols., 2022c**; **Plaza y cols., 2019**), podría relacionarse con un grado de β-oxidación menor en tejidos como el corazón y el músculo esquelético, capaz de promover la acumulación ectópica de lípidos y su consiguiente lipotoxicidad (**Breslow y cols., 1999; Lee y cols., 2002; Minokoshi y cols., 2002; Plaza y cols., 2019**). El mecanismo por el cual la dieta SOLF ejerce esta alteración endocrina es desconocido, pero estudios que se están llevando a cabo actualmente en el laboratorio sugieren que la unión del ácido láurico, presente en la dieta, al factor de transcripción **PPARγ** (**Manuscrito en preparación**) podría ser la responsable. Cuando analizamos el efecto de las dietas sobre la morfología del TAB, observamos que mientras UOLF favorece la hipertrofia de los adipocitos, SOLF apenas eleva su tamaño, aunque ambas dietas reprimen la expresión de genes implicados en la diferenciación adipocitaria (**Fernández-Felipe y cols., 2022a**). Sin embargo, no hemos observado con ninguna de las dietas la activación de la respuesta inflamatoria ni fibrosis del tejido, frecuentes en la obesidad inducida por el consumo de HFDs (**Marcelin y cols., 2022**). Así, podemos asumir que la alteración endocrina del TAB es independiente de inflamación y fibrosis y proponemos que la diferencia en la expresión de leptina podría ser el elemento distintivo generado por las dietas en el TAB.

A pesar de que la dieta SOLF no aumenta la producción de leptina, si bloquea la activación por leptina exógena de las vías STAT3, Akt y AMPK en el TAB-Vis (Figura 13), mientras que en el TAB-Sc causa, además, un aumento de los niveles basales de pSTAT3, pAkt y pAMPK (**Fernández-Felipe y cols., 2022a**). En este caso, nos preguntamos si la aparente falta de respuesta a leptina se debe estrictamente a la desensibilización del receptor o es consecuencia del aumento de la fosforilación de STAT3, Akt y AMPK causado por la dieta, capaz de enmascarar el efecto de la leptina. Puesto que estas vías de señalización participan en la diferenciación y proliferación de adipocitos (**Deng y cols., 2000; Habinowski y Witters, 2001; Taniguchi y cols., 2006**), la ausencia de respuesta a leptina en los animales SOLF podría reducir la adaptación del TAB a la sobrecarga de lípidos de la dieta, ya que la leptina

limita la expansibilidad del tejido adiposo (**Ramsay, 2005**); por el contrario, la hiperleptinemia asociada a la obesidad, y la consecuente resistencia a la hormona, promueven la hipertrofia de los adipocitos, favoreciéndose la inflamación del tejido (**Bogacka y cols., 2004; Ramsay y Richards, 2004**). En este contexto, nuestros datos sugieren que la hipertrofia del TAB observada en los animales SOLF podría deberse a la disminución del efecto autocrino de la leptina, que como ya hemos comentado anteriormente, podría estar relacionada con el efecto directo de los ácidos grasos de la dieta sobre la funcionalidad de su receptor, como describen otros autores (**Gruzdeva y cols., 2019**).

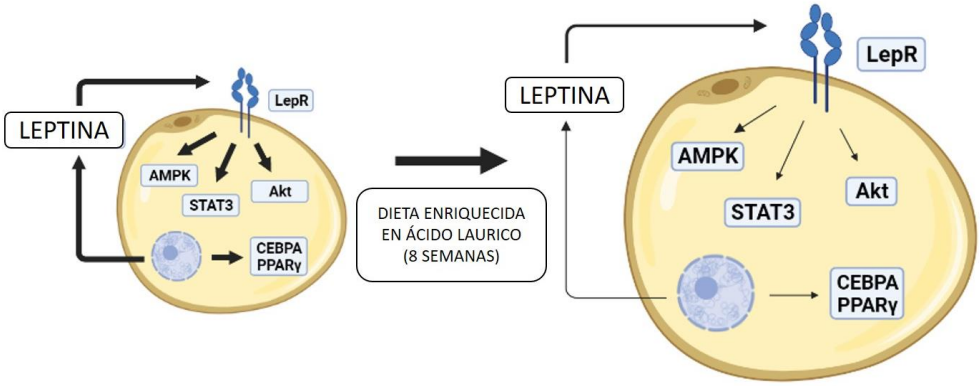


Figura 13. Representación gráfica de los efectos de SOLF sobre el TAB visceral. Imagen modificada de Fernández-Felipe y cols., 2022a.

Teniendo en cuenta que los ácidos grasos plasmáticos derivan principalmente de la lipólisis en el TAB (**Hellmuth y cols., 2013**), decidimos analizar la composición en ácidos grasos del TAB-Sc y del TAB-Vis. Observamos que, en comparación con los animales control, el TAB de los animales UOLF acumula principalmente ácido oleico, mientras que el de los SOLF contiene sobre todo ácidos grasos saturados, mayoritariamente ácidos laúrico, palmítico y mirístico. Estos cambios podrían ser responsables de las diferencias morfológicas y funcionales observadas entre SOLF y UOLF. De hecho, los ácidos grasos presentan propiedades biofísicas que dependen de la longitud de su cadena y del número de insaturaciones, de forma que las grasas saturadas se empaquetan más que las mono- y poliinsaturadas, dando lugar a vesículas lipídicas más pequeñas y densas (**Bavelaar y Beynen, 2003; Knothe y Dunn, 2009**). Esta circunstancia podría explicar el menor grado de hipertrofia del TAB de los animales SOLF. Además, al tratarse de un tejido

adiposo menos fluido que el de los animales UOLF, se podrían modificar sus propiedades mecánicas (**Hsieh y cols., 2019; Shoham y cols., 2014**), lo que puede alterar la funcionalidad de sus receptores de membrana (**Shoham y Gefen, 2012**), incluido el RLep (**Phillips y cols., 2009**). A partir de todas estas consideraciones, sugerimos que los cambios generados por las dietas SOLF y UOLF en el TAB podrían estar relacionados con el efecto bioquímico de los ácidos grasos sobre los adipocitos (mediados, al menos parcialmente por PPARγ) y con cambios biofísicos ligados a la composición del TAB.

Los efectos perjudiciales de las dietas SOLF y UOLF no se limitan al TAB, sino que afectan también al SNC. Los estudios llevados a cabo en esta Tesis Doctoral demuestran que las dietas ricas en grasas saturadas son más perjudiciales para la plasticidad sináptica y la memoria dependiente de hipocampo que las dietas con alto contenido en grasas monoinsaturadas (Figura 14). Nuestros resultados muestran que ambas dietas afectan a la señalización del RLep en el hipocampo, aunque de forma diferente; mientras SOLF aumenta la fosforilación de STAT3, UOLF desensibiliza las vías de Akt y AMPK (**Fernández-Felipe y cols., 2023, Anexo I**). Cabe recalcar que, a pesar de existir estas alteraciones en la respuesta a leptina, ésta hormona sólo está elevada en los animales UOLF, lo que sugiere que la alteración de la funcionalidad del RLep es independiente de la hiperleptinemia, al igual que ocurre en el TAB. Además, se puede especular que, al igual que ocurre con el TAB, las dietas podrían modificar la composición de la membrana celular de las neuronas, lo que podría estar relacionado con la modificación de la señalización del RLep (**Heshka y Jones, 2001**) y de los mecanismos de plasticidad sináptica (**Egawa y cols., 2016; Westra y cols., 2021**). Alternativamente, las alteraciones del metabolismo lipídico causadas por la dieta también podrían tener un papel relevante en los cambios que se observan (**Kennedy y cols., 2009; Unger, 2002**). En este sentido, un trabajo en curso en nuestro laboratorio muestra que las dietas provocan cambios en el perfil de ceramidas en el hipocampo, cuyo significado está pendiente de evaluación.

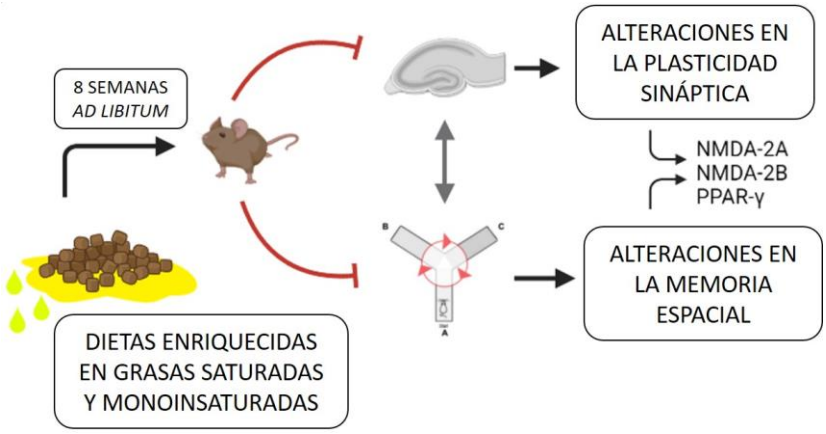


Figura 14. Representación gráfica del efecto SOLF y UOLF sobre el hipocampo y la memoria espacial dependiente de hipocampo. *Adaptada de* ***Fernández-Felipe y cols., 2021****.*

En relación con el efecto inhibitorio de la dieta SOLF sobre la LTP en el hipocampo, nuestros datos son coherentes con estudios previos que demuestran que los ácidos grasos saturados, como el ácido palmítico, alteran la LTP (**Contreras y cols., 2017**) y deterioran la memoria espacial (**Ge y cols., 2010; Lüscher y Malenka, 2012; Malenka, 1994**). Además la LTP es dependiente de la fosforilación de STAT3 (**Nicolas y cols., 2012**) y en nuestro modelo vemos que SOLF altera la activación de este factor de transcripción. Por otra parte, hemos observado que la ingesta de SOLF altera los niveles de aminoácidos esenciales en la transmisión sináptica hipocampal, aumentando las cantidades de L-Glu y L-Asp, y disminuyendo los de L-Gln y GABA (**Fernández-Felipe y cols., 2022b, Anexo II**) (Figura 15), tal y como ocurre en la obesidad (**Labban y cols., 2020; Lizarbe y cols., 2019; Ribeiro y cols., 2018**). Estos cambios podrían afectar negativamente a la plasticidad sináptica hipocampal y la memoria (**Lizarbe y cols., 2019; Valladolid-Acebes y cols., 2012**). Además, SOLF disminuye la expresión de PPARγ, un regulador esencial del metabolismo de aminoácidos en el SNC (**Aleshin y Reiser, 2013; Youssef y Badr, 2004**). Así, un metabolismo defectuoso de los aminoácidos neurotransmisores, junto a la desregulación en la señalización de leptina, podrían ser los responsables del deterioro de los mecanismos de plasticidad sináptica hipocampal causados por esta dieta.

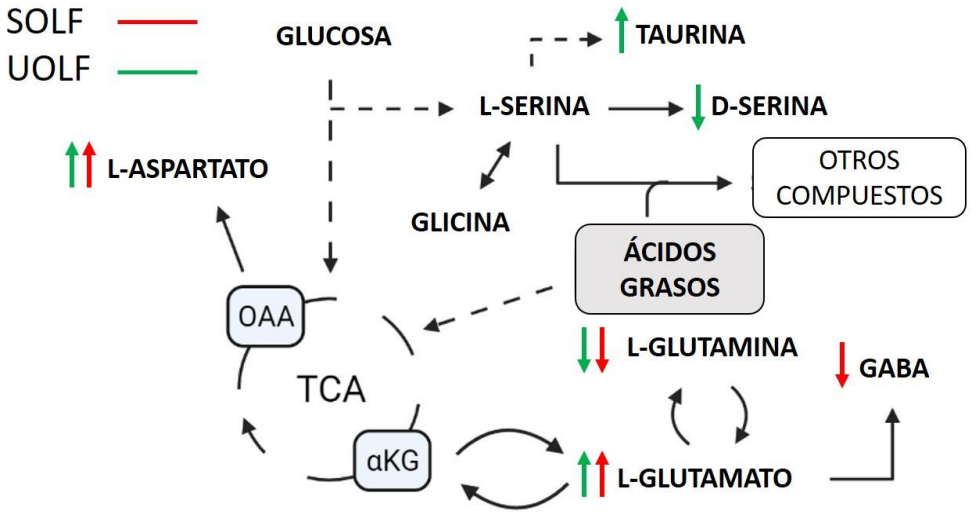


Figura 15. Representación gráfica de las rutas de síntesis de los aminoácidos que participan en las sinapsis hipocampales. *En flechas verdes se muestra el efecto del consumo de la dieta UOLF y el rojo el efecto del consumo de la dieta SOLF.*

Nuestros resultados muestran que la dieta UOLF también tiene importantes consecuencias en la plasticidad sináptica en el hipocampo. Al igual que en estudios anteriores, empleando HFDs convencionales (**Hao y cols., 2016; Hwang y cols., 2010; Valladolid-Acebes y cols., 2012**), la dieta UOLF abole la LTD inducida por NMDA, además de bloquear el mantenimiento de la LTP. De hecho, en el hipocampo de los animales UOLF se observa una desensibilización de la vía Akt en respuesta a leptina, cuya activación es esencial tanto para la LTP como para la LTD (**Peineau y cols., 2007; Zhao y cols., 2019**). Además, el consumo de UOLF modifica los niveles de aminoácidos excitadores de forma similar a SOLF, aunque también disminuye los niveles de D-serina (**Fernández-Felipe y cols., 2022b, Anexo II**), un aminoácido clave en la funcionalidad de los receptores de NMDA (**Balan y cols., 2009; Neame y cols., 2019**) y esencial en el mantenimiento de la LTP y LTD (**Panatier y cols., 2006**) (Figura 15). De esta manera, tanto los desajustes en la señalización de la leptina como la alteración en los niveles de neurotransmisores, particularmente de D-serina, podrían estar detrás de los efectos observados sobre la plasticidad sináptica por esta dieta.

El consumo de las dietas SOLF y UOLF también afecta al hipotálamo, donde la dieta SOLF desensibiliza la fosforilación de Akt y AMPK en respuesta a leptina exógena (**Fernández-Felipe y cols., 2022c**) (Figura 16). Está descrito

que la inflamación y la gliosis en el hipotálamo están asociadas al desarrollo de resistencia a leptina e insulina (**De Souza y cols., 2005; Douglass y cols., 2017**). En nuestro modelo, a pesar no detectarse elevación de citoquinas proinflamatorias, sí se observa un aumento de GFAP, que es un marcador de gliosis. Sin embargo, no podemos determinar si la falta de respuesta a leptina se debe a la gliosis o es un acontecimiento concomitante al consumo de los ácidos grasos, ya que las grasas *per se* pueden inducir resistencia a leptina (**Cheng y cols., 2015; Oh y cols., 2013**). Aun así, a diferencia de lo que observan otros grupos (**Koch y cols., 2014; Münzberg y cols., 2004**), la resistencia a leptina encontrada en el hipotálamo de los animales SOLF podría deberse a la presencia particular de ácido láurico o a las alteraciones en el metabolismo lipídico causadas por la dieta (**Hirabayashi y Furuya, 2008; Movsesyan y cols., 2002**).

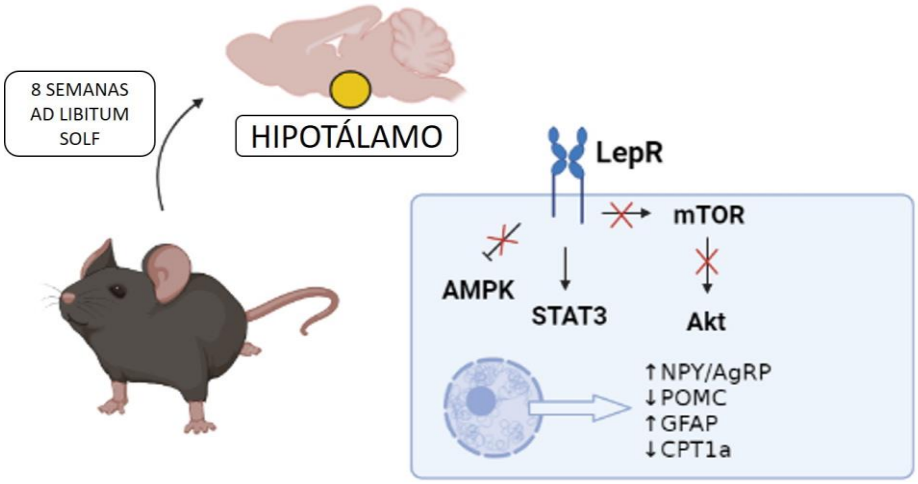


***Figura 16. Representación gráfica del efecto de la dieta SOLF en el hipotálamo*.**

CONCLUSIONES

1. Las dietas SOLF y UOLF aumenta la cantidad de tejido adiposo blanco (TAB) pero la expresión de leptina y la leptina plasmática solo aumentan en los animales UOLF.

2. La dieta SOLF activa las vías de STAT3, Akt y AMPK en el TAB subcutáneo, donde no hay hipertrofia, mientras que desensibiliza el receptor de leptina en el TAB visceral, donde sí existe hipertrofia.

3. Ambas dietas modifican la composición del TAB, encontrando mayor cantidad de ácido láurico, palmítico y mirístico en el de los animales que ingieren SOLF y ácido oleico en el de aquellos que ingieren UOLF. Estas diferencias podrían alterar las propiedades biofísicas del TAB e influir negativamente en la señalización del receptor de leptina.

4. La dieta SOLF deteriora la transmisión sináptica basal y daña los mecanismos de plasticidad sináptica en el hipocampo, además de deteriorar la memoria espacial dependiente de hipocampo.

5. La dieta UOLF impide el mantenimiento de la LTP y la LTD, sin alterar la transmisión sináptica basal ni la memoria espacial dependiente de hipocampo.

6. Ambas dietas elevan los niveles de L-glutamato y disminuyen los de L-glutamina. Sin embargo, sólo la dieta UOLF disminuye los niveles de D-serina. Estas alteraciones podrían contribuir al efecto de las dietas sobre la plasticidad sináptica.

7. Ambas dietas alteran la señalización del receptor de leptina en el hipocampo. Mientras que SOLF aumenta la fosforilación basal de STAT3, UOLF desensibiliza las vías de Akt y AMPK.

8. En el hipotálamo, sólo la dieta SOLF desensibiliza las vías Akt-AMPK y aumenta marcadores de gliosis, indicando que esta área cerebral y su señalización del receptor de leptina son sensibles al consumo de grasas saturadas.

9. En conjunto, ambas dietas modulan el eje tejido adiposo-cerebro, pero lo hacen de forma diferente**.** Una dieta enriquecida en ácido láurico y palmítico (SOLF) modifica la función endocrina del tejido adiposo y la función hipocampal, alterando al mismo tiempo la señalización del receptor de leptina en ausencia de hiperleptinemia. El consumo de una dieta rica en ácido oleico (UOLF) tiene también efectos perjudiciales sobre la funcionalidad del hipocampo, que en este caso podrían atribuirse a la presencia de hiperleptinemia. Así, los ácidos grasos presentes en la dieta pueden ser, al menos parcialmente, responsables de los efectos perjudiciales causados por ellas independiente de la elevación en la producción de leptina**.**

BIBLIOGRAFÍA

Adams, M.M., Smith, T.D., Moga, D., Gallagher, M., Wang, Y., Wolfe, B.B., Rapp, P.R., Morrison, J.H., 2001. Hippocampal dependent learning ability correlates with N-methyl-D-aspartate (NMDA) receptor levels in CA3 neurons of young and aged rats. J. Comp. Neurol. 432, 230–243. https://doi.org/10.1002/cne.1099

Aguilar-Valles, A., Inoue, W., Rummel, C., Luheshi, G.N., 2015. Obesity, adipokines and neuroinflammation. Neuropharmacology 96, 124–134. https://doi.org/10.1016/j.neuropharm.2014.12.023

Ahima, R.S., 2006. Adipose tissue as an endocrine organ. Obesity (Silver Spring). 14 Suppl 5, 242S-249S. https://doi.org/10.1038/oby.2006.317

Ahima, R.S., Flier, J.S., 2000. Leptin. Annu. Rev. Physiol. 62, 413–437. https://doi.org/10.1146/annurev.physiol.62.1.413

Ahima, R.S., Saper, C.B., Flier, J.S., Elmquist, J.K., 2000. Leptin regulation of neuroendocrine systems. Front. Neuroendocrinol. 21, 263–307. https://doi.org/10.1006/frne.2000.0197

Aleshin, S., Reiser, G., 2013. Role of the peroxisome proliferator-activated receptors (PPAR)-α, β/δ and γ triad in regulation of reactive oxygen species signaling in brain. Biol. Chem. 394, 1553–1570. https://doi.org/10.1515/hsz-2013-0215

Amaral, D.G., Witter, M.P., 1989. The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience 31, 571–591. https://doi.org/10.1016/0306-4522(89)90424-7

Amtul, Z., Westaway, D., Cechetto, D.F., Rozmahel, R.F., 2011. Oleic acid ameliorates amyloidosis in cellular and mouse models of alzheimer's disease. Brain Pathol. 21, 321–329. https://doi.org/10.1111/j.1750-3639.2010.00449.x

Arner, P., 1997. Regional adipocity in man. J. Endocrinol. 155, 191–192. https://doi.org/10.1677/joe.0.1550191

Aune, D., Ursin, G., Veierød, M.B., 2009. Meat consumption and the risk of type 2 diabetes: a systematic review and meta-analysis of cohort studies. Diabetologia 52, 2277–2287. https://doi.org/10.1007/s00125-009-1481-x

Bak, L.K., Schousboe, A., Waagepetersen, H.S., 2006. The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. J. Neurochem. 98, 641–653. https://doi.org/10.1111/j.1471-4159.2006.03913.x

Balan, L., Foltyn, V.N., Zehl, M., Dumin, E., Dikopoltsev, E., Knoh, D., Ohno, Y., Kihara, A., Jensen, O.N., Radzishevsky, I.S., Wolosker, H., 2009. Feedback inactivation of D-serine synthesis by NMDA receptor-elicited translocation of serine racemase to the membrane.

Proc. Natl. Acad. Sci. U. S. A. 106, 7589–7594. https://doi.org/10.1073/pnas.0809442106

Balu, D.T., Coyle, J.T., 2011. Neuroplasticity signaling pathways linked to the pathophysiology of schizophrenia. Neurosci. Biobehav. Rev. 35, 848–870. https://doi.org/10.1016/j.neubiorev.2010.10.005

Banks, W.A., Kastin, A.J., Huang, W., Jaspan, J.B., Maness, L.M., 1996. Leptin enters the brain by a saturable system independent of insulin. Peptides 17, 305–311. https://doi.org/10.1016/0196-9781(96)00025-3

Bavelaar, F.J., Beynen, A.C., 2003. Relationships between dietary fatty acid composition and either melting point or fatty acid profile of adipose tissue in broilers. Meat Sci. 64, 133–140. https://doi.org/https://doi.org/10.1016/S0309-1740(02)00167-5

Bazan, J.F., 1989. A novel family of growth factor receptors: a common binding domain in the growth hormone, prolactin, erythropoietin and IL-6 receptors, and the p75 IL-2 receptor beta-chain. Biochem. Biophys. Res. Commun. 164, 788–795. https://doi.org/10.1016/0006-291x(89)91528-3

Beart, P.M., O'Shea, R.D., 2007. Transporters for L-glutamate: an update on their molecular pharmacology and pathological involvement. Br. J. Pharmacol. 150, 5–17. https://doi.org/10.1038/sj.bjp.0706949

Beltrán-Castillo, S., Eugenín, J., Von Bernhardi, R., 2018. Impact of aging in microglia-mediated D-serine balance in the CNS. Mediators Inflamm. 2018. https://doi.org/10.1155/2018/7219732

Bento-Abreu, A., Tabernero, A., Medina, J.M., 2007. Peroxisome proliferator-activated receptor-alpha is required for the neurotrophic effect of oleic acid in neurons. J. Neurochem. 103, 871–881. https://doi.org/10.1111/j.1471-4159.2007.04807.x

Billington, C.J., Briggs, J.E., Grace, M., Levine, A.S., 1991. Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. Am. J. Physiol. 260, R321-7. https://doi.org/10.1152/ajpregu.1991.260.2.R321

Bjørbaek, C., Kahn, B.B., 2004. Leptin signaling in the central nervous system and the periphery. Recent Prog. Horm. Res. 59, 305–331. https://doi.org/10.1210/rp.59.1.305

Bjørbaek, C., Uotani, S., da Silva, B., Flier, J.S., 1997. Divergent signaling capacities of the long and short isoforms of the leptin receptor. J. Biol. Chem. 272, 32686–32695. https://doi.org/10.1074/jbc.272.51.32686

Blüher, M., 2020. Metabolically Healthy Obesity. Endocr. Rev. 41. https://doi.org/10.1210/endrev/bnaa004

Bogacka, I., Roane, D.S., Xi, X., Zhou, J., Li, B., Ryan, D.H., Martin, R.J., 2004. Expression levels of genes likely involved in glucose-sensing in the obese Zucker rat brain. Nutr.

Neurosci. 7, 67–74. https://doi.org/10.1080/10284150410001710401

Bolaños, J.P., 2016. Bioenergetics and redox adaptations of astrocytes to neuronal activity. J. Neurochem. 139, 115–125. https://doi.org/10.1111/jnc.13486

Bornstein, S.R., Abu-Asab, M., Glasow, A., Päth, G., Hauner, H., Tsokos, M., Chrousos, G.P., Scherbaum, W.A., 2000. Immunohistochemical and ultrastructural localization of leptin and leptin receptor in human white adipose tissue and differentiating human adipose cells in primary culture. Diabetes 49, 532–538. https://doi.org/10.2337/diabetes.49.4.532

Bornstein, S.R., Uhlmann, K., Haidan, A., Ehrhart-Bornstein, M., Scherbaum, W.A., 1997. Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland: leptin inhibits cortisol release directly. Diabetes 46, 1235–1238. https://doi.org/10.2337/diab.46.7.1235

Boulter, J., Hollmann, M., O'Shea-Greenfield, A., Hartley, M., Deneris, E., Maron, C., Heinemann, S., 1990. Molecular cloning and functional expression of glutamate receptor subunit genes. Science 249, 1033–1037. https://doi.org/10.1126/science.2168579

Bowden, J.B., Abraham, W.C., Harris, K.M., 2012. Differential effects of strain, circadian cycle, and stimulation pattern on LTP and concurrent LTD in the dentate gyrus of freely moving rats. Hippocampus 22, 1363–1370. https://doi.org/10.1002/hipo.20972

Brager, D.H., Cai, X., Thompson, S.M., 2003. Activity-dependent activation of presynaptic protein kinase C mediates post-tetanic potentiation. Nat. Neurosci. 6, 551–552. https://doi.org/10.1038/nn1067

Breslow, M.J., Min-Lee, K., Brown, D.R., Chacko, V.P., Palmer, D., Berkowitz, D.E., 1999. Effect of leptin deficiency on metabolic rate in ob/ob mice. Am. J. Physiol. 276, E443-9. https://doi.org/10.1152/ajpendo.1999.276.3.E443

Budreviciute, A., Damiati, S., Sabir, D.K., Onder, K., Schuller-Goetzburg, P., Plakys, G., Katileviciute, A., Khoja, S., Kodzius, R., 2020. Management and Prevention Strategies for Non-communicable Diseases (NCDs) and Their Risk Factors. Front. public Heal. 8, 574111. https://doi.org/10.3389/fpubh.2020.574111

Buonomano, D. V, Merzenich, M.M., 1998. Cortical plasticity: from synapses to maps. Annu. Rev. Neurosci. 21, 149–186. https://doi.org/10.1146/annurev.neuro.21.1.149

Cammisotto, P.G., Gélinas, Y., Deshaies, Y., Bukowiecki, L.J., 2003. Regulation of leptin secretion from white adipocytes by free fatty acids. Am. J. Physiol. Endocrinol. Metab. 285, E521-6. https://doi.org/10.1152/ajpendo.00052.2003

Caprio, S., Tamborlane, W. V, Silver, D., Robinson, C., Leibel, R., McCarthy, S., Grozman, A., Belous, A., Maggs, D., Sherwin, R.S., 1996. Hyperleptinemia: an early sign of juvenile obesity. Relations to body fat depots and insulin concentrations. Am. J. Physiol. 271,

E626-30. https://doi.org/10.1152/ajpendo.1996.271.3.E626

Carpenter-Hyland, E.P., Chandler, L.J., 2007. Adaptive plasticity of NMDA receptors and dendritic spines: implications for enhanced vulnerability of the adolescent brain to alcohol addiction. Pharmacol. Biochem. Behav. 86, 200–208. https://doi.org/10.1016/j.pbb.2007.01.016

Castanon, N., Lasselin, J., Capuron, L., 2014. Neuropsychiatric comorbidity in obesity: role of inflammatory processes. Front. Endocrinol. (Lausanne). 5, 74. https://doi.org/10.3389/fendo.2014.00074

Castanon, N., Luheshi, G., Layé, S., 2015. Role of neuroinflammation in the emotional and cognitive alterations displayed by animal models of obesity. Front. Neurosci. 9, 229. https://doi.org/10.3389/fnins.2015.00229

Cheng, A., Uetani, N., Simoncic, P.D., Chaubey, V.P., Lee-Loy, A., McGlade, C.J., Kennedy, B.P., Tremblay, M.L., 2002. Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. Dev. Cell 2, 497–503. https://doi.org/10.1016/s1534-5807(02)00149-1

Cheng, L., Yu, Y., Szabo, A., Wu, Y., Wang, H., Camer, D., Huang, X.-F., 2015a. Palmitic acid induces central leptin resistance and impairs hepatic glucose and lipid metabolism in male mice. J. Nutr. Biochem. 26, 541–548. https://doi.org/10.1016/j.jnutbio.2014.12.011

Cheng, L., Yu, Y., Szabo, A., Wu, Y., Wang, H., Camer, D., Huang, X.F., 2015b. Palmitic acid induces central leptin resistance and impairs hepatic glucose and lipid metabolism in male mice. J. Nutr. Biochem. 26, 541–548. https://doi.org/10.1016/j.jnutbio.2014.12.011

Clayton, D.A., Mesches, M.H., Alvarez, E., Bickford, P.C., Browning, M.D., 2002. A hippocampal NR2B deficit can mimic age-related changes in long-term potentiation and spatial learning in the Fischer 344 rat. J. Neurosci. 22, 3628–3637. https://doi.org/10.1523/JNEUROSCI.22-09-03628.2002

Collingridge, G.L., Peineau, S., Howland, J.G., Wang, Y.T., 2010. Long-term depression in the CNS. Nat. Rev. Neurosci. 11, 459–473. https://doi.org/10.1038/nrn2867

Considine, R. V, Sinha, M.K., Heiman, M.L., Kriauciunas, A., Stephens, T.W., Nyce, M.R., Ohannesian, J.P., Marco, C.C., McKee, L.J., Bauer, T.L., 1996. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334, 292–295. https://doi.org/10.1056/NEJM199602013340503

Contreras, A., Del Rio, D., Martínez, A., Gil, C., Morales, L., Ruiz-Gayo, M., Del Olmo, N., 2017. Inhibition of hippocampal long-term potentiation by high-fat diets: Is it related to an effect of palmitic acid involving glycogen synthase kinase-3? Neuroreport 28, 354–359. https://doi.org/10.1097/WNR.0000000000000774

Cordain, L., Eaton, S.B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B.A., O'Keefe, J.H., Brand-Miller, J., 2005. Origins and evolution of the Western diet: health implications for the 21st century. Am. J. Clin. Nutr. 81, 341–354. https://doi.org/10.1093/ajcn.81.2.341

Dagon, Y., Hur, E., Zheng, B., Wellenstein, K., Cantley, L.C., Kahn, B.B., 2012. p70S6 kinase phosphorylates AMPK on serine 491 to mediate leptin's effect on food intake. Cell Metab. 16, 104–112. https://doi.org/10.1016/j.cmet.2012.05.010

Dallman, M.F., 2010. Stress-induced obesity and the emotional nervous system. Trends Endocrinol. Metab. 21, 159–165. https://doi.org/10.1016/j.tem.2009.10.004

Dalvi, P.S., Chalmers, J.A., Luo, V., Han, D.-Y., Wellhauser, L., Liu, Y., Tran, D.Q., Castel, J., Luquet, S., Wheeler, M.B., Belsham, D.D., 2017. High fat induces acute and chronic inflammation in the hypothalamus: effect of high-fat diet, palmitate and TNF-α on appetite-regulating NPY neurons. Int. J. Obes. (Lond). 41, 149–158. https://doi.org/10.1038/ijo.2016.183

Danbolt, N.C., 2001. Glutamate uptake. Prog. Neurobiol. 65, 1–105. https://doi.org/10.1016/s0301-0082(00)00067-8

Davidson, T.L., Kanoski, S.E., Schier, L.A., Clegg, D.J., Benoit, S.C., 2007. A potential role for the hippocampus in energy intake and body weight regulation. Curr. Opin. Pharmacol. 7, 613–616. https://doi.org/10.1016/j.coph.2007.10.008

Davidson, T.L., Kanoski, S.E., Walls, E.K., Jarrard, L.E., 2005. Memory inhibition and energy regulation. Physiol. Behav. 86, 731–746. https://doi.org/10.1016/j.physbeh.2005.09.004

De Felice, F.G., Ferreira, S.T., 2014. Inflammation, defective insulin signaling, and mitochondrial dysfunction as common molecular denominators connecting type 2 diabetes to Alzheimer disease. Diabetes 63, 2262–2272. https://doi.org/10.2337/db13-1954

de la Monte, S.M., Wands, J.R., 2008. Alzheimer's disease is type 3 diabetes-evidence reviewed. J. Diabetes Sci. Technol. 2, 1101–1113. https://doi.org/10.1177/193229680800200619

De Souza, C.T., Araujo, E.P., Bordin, S., Ashimine, R., Zollner, R.L., Boschero, A.C., Saad, M.J.A., Velloso, L.A., 2005. Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology 146, 4192–4199. https://doi.org/10.1210/en.2004-1520

del Mar Romero, M., Fernández-López, J.A., Esteve, M., Alemany, M., 2009. Site-related white adipose tissue lipid-handling response to oleoyl-estrone treatment in overweight male rats. Eur. J. Nutr. 48, 291–299. https://doi.org/10.1007/s00394-009-0013-2

Deng, C., Moinat, M., Curtis, L., Nadakal, A., Preitner, F., Boss, O., Assimacopoulos-Jeannet,

F., Seydoux, J., Giacobino, J.P., 1997. Effects of beta-adrenoceptor subtype stimulation on obese gene messenger ribonucleic acid and on leptin secretion in mouse brown adipocytes differentiated in culture. Endocrinology 138, 548–552. https://doi.org/10.1210/endo.138.2.4922

Deng, J., Hua, K., Lesser, S.S., Harp, J.B., 2000. Activation of signal transducer and activator of transcription-3 during proliferative phases of 3T3-L1 adipogenesis. Endocrinology 141, 2370–2376. https://doi.org/10.1210/endo.141.7.7551

Deng, P.-Y., Sojka, D., Klyachko, V.A., 2011. Abnormal presynaptic short-term plasticity and information processing in a mouse model of fragile X syndrome. J. Neurosci. 31, 10971–10982. https://doi.org/10.1523/JNEUROSCI.2021-11.2011

Devos, R., Guisez, Y., Van der Heyden, J., White, D.W., Kalai, M., Fountoulakis, M., Plaetinck, G., 1997. Ligand-independent dimerization of the extracellular domain of the leptin receptor and determination of the stoichiometry of leptin binding. J. Biol. Chem. 272, 18304–18310. https://doi.org/10.1074/jbc.272.29.18304

Diamond, D.M., Park, C.R., Campbell, A.M., Woodson, J.C., 2005. Competitive interactions between endogenous LTD and LTP in the hippocampus underlie the storage of emotional memories and stress-induced amnesia. Hippocampus 15, 1006–1025. https://doi.org/10.1002/hipo.20107

Dickson, S.L., Chowen, J.A., 2020. Neuroscience of obesity. Neuroscience. https://doi.org/10.1016/j.neuroscience.2020.09.047

Dinel, A.-L., André, C., Aubert, A., Ferreira, G., Layé, S., Castanon, N., 2011. Cognitive and emotional alterations are related to hippocampal inflammation in a mouse model of metabolic syndrome. PLoS One 6, e24325. https://doi.org/10.1371/journal.pone.0024325

Ding, X., Ma, N., Nagahama, M., Yamada, K., Semba, R., 2011. Localization of D-serine and serine racemase in neurons and neuroglias in mouse brain. Neurol. Sci. Off. J. Ital. Neurol. Soc. Ital. Soc. Clin. Neurophysiol. 32, 263–267. https://doi.org/10.1007/s10072-010-0422-2

Douglass, J.D., Dorfman, M.D., Fasnacht, R., Shaffer, L.D., Thaler, J.P., 2017. Astrocyte IKKβ/NF-κB signaling is required for diet-induced obesity and hypothalamic inflammation. Mol. Metab. 6, 366–373. https://doi.org/10.1016/j.molmet.2017.01.010

Durakoglugil, M., Irving, A.J., Harvey, J., 2005. Leptin induces a novel form of NMDA receptor-dependent long-term depression. J. Neurochem. 95, 396–405. https://doi.org/10.1111/j.1471-4159.2005.03375.x

Dutia, R., Meece, K., Dighe, S., Kim, A.J., Wardlaw, S.L., 2012. β-Endorphin antagonizes the effects of α-MSH on food intake and body weight. Endocrinology 153, 4246–4255. https://doi.org/10.1210/en.2012-1166

Dzamko, N., van Denderen, B.J.W., Hevener, A.L., Jørgensen, S.B., Honeyman, J., Galic, S., Chen, Z.-P., Watt, M.J., Campbell, D.J., Steinberg, G.R., Kemp, B.E., 2010. AMPK beta1 deletion reduces appetite, preventing obesity and hepatic insulin resistance. J. Biol. Chem. 285, 115–122. https://doi.org/10.1074/jbc.M109.056762

Eaton, S.B., Konner, M., Shostak, M., 1988. Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective. Am. J. Med. 84, 739–749. https://doi.org/10.1016/0002-9343(88)90113-1

Egawa, J., Pearn, M.L., Lemkuil, B.P., Patel, P.M., Head, B.P., 2016. Membrane lipid rafts and neurobiology: age-related changes in membrane lipids and loss of neuronal function. J. Physiol. 594, 4565–4579. https://doi.org/10.1113/JP270590

Elmquist, J.K., Maratos-Flier, E., Saper, C.B., Flier, J.S., 1998. Unraveling the central nervous system pathways underlying responses to leptin. Nat. Neurosci. 1, 445–450. https://doi.org/10.1038/2164

Esteve Ràfols, M., 2014. Adipose tissue: Cell heterogeneity and functional diversity. Endocrinol. y Nutr. (English Ed. 61, 100–112. https://doi.org/10.1016/j.endoen.2014.02.001

Falkowska, A., Gutowska, I., Goschorska, M., Nowacki, P., Chlubek, D., Baranowska-Bosiacka, I., 2015. Energy metabolism of the brain, including the cooperation between astrocytes and neurons, especially in the context of glycogen metabolism. Int. J. Mol. Sci. 16, 25959–25981. https://doi.org/10.3390/ijms161125939

Fan, W., Boston, B.A., Kesterson, R.A., Hruby, V.J., Cone, R.D., 1997. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385, 165–168. https://doi.org/10.1038/385165a0

Farmer, S.R., 2006. Transcriptional control of adipocyte formation. Cell Metab. 4, 263–273. https://doi.org/10.1016/j.cmet.2006.07.001

Farr, O.M., Gavrieli, A., Mantzoros, C.S., 2015. Leptin applications in 2015: what have we learned about leptin and obesity? Curr. Opin. Endocrinol. Diabetes. Obes. 22, 353–359. https://doi.org/10.1097/MED.0000000000000184

Farr, S.A., Banks, W.A., Morley, J.E., 2006. Effects of leptin on memory processing. Peptides 27, 1420–1425. https://doi.org/10.1016/j.peptides.2005.10.006

Fernández-Felipe, J., López, L.L., Cano, V., Sánchez-Hita, E., Belén Sanz, A., Chowen, J.A., Del Olmo, N., Ruiz-Gayo, M., Merino, B., 2023. Regional specific effect of saturated vs unsaturated fat on leptin receptor signalling in mice brain areas regulating feeding. Neurosci. Lett. 793, 136996. https://doi.org/https://doi.org/10.1016/j.neulet.2022.136996

Fernández-Felipe, J., Merino, B., Sanz-Martos, A.B., Plaza, A., Contreras, A., Naranjo, V., Morales, L., Chowen, J.A., Cano, V., Ruiz-Gayo, M., Del Olmo, N., 2021. Saturated and

unsaturated fat diets impair hippocampal glutamatergic transmission in adolescent mice. Psychoneuroendocrinology 105429. https://doi.org/10.1016/j.psyneuen.2021.105429

Fernández-Felipe, J., Plaza, A., Domínguez, G., Pérez-Castells, J., Cano, V., Cioni, F., Del Olmo, N., Ruiz-Gayo, M., Merino, B., 2022a. Effect of Lauric vs. Oleic Acid-Enriched Diets on Leptin Autoparacrine Signalling in Male Mice. Biomedicines 10. https://doi.org/10.3390/biomedicines10081864

Fernández-Felipe, J., Sanz-Martos, A.B., Marcos, A., Lorenzo, M.P., Cano, V., Merino, B., Ambrosio, E., Del Olmo, N., Ruiz-Gayo, M., 2022b. Saturated and unsaturated triglyceride-enriched diets modify amino acid content in the mice hippocampus. Neurosci. Lett. 136972. https://doi.org/https://doi.org/10.1016/j.neulet.2022.136972

Fernández-Felipe, J., Valencia-Avezuela, M., Merino, B., Somoza, B., Cano, V., Sanz-Martos, A.B., Frago, L.M., Fernández-Alfonso, M.S., Ruiz-Gayo, M., Chowen, J.A., 2022c. Effects of saturated versus unsaturated fatty acids on metabolism, gliosis, and hypothalamic leptin sensitivity in male mice. Nutr. Neurosci. 1–14. https://doi.org/10.1080/1028415X.2022.2029294

Ferreira, S.T., Clarke, J.R., Bomfim, T.R., De Felice, F.G., 2014. Inflammation, defective insulin signaling, and neuronal dysfunction in Alzheimer's disease. Alzheimers. Dement. 10, S76-83. https://doi.org/10.1016/j.jalz.2013.12.010

Flament-Durand, J., 1980. The hypothalamus: anatomy and functions. Acta Psychiatr. Belg. 80, 364–375.

Fliers, E., Kreier, F., Voshol, P.J., Havekes, L.M., Sauerwein, H.P., Kalsbeek, A., Buijs, R.M., Romijn, J.A., 2003. White adipose tissue: getting nervous. J. Neuroendocrinol. 15, 1005–1010. https://doi.org/10.1046/j.1365-2826.2003.01096.x

Forny-Germano, L., De Felice, F.G., Vieira, M.N. do N., 2018. The Role of Leptin and Adiponectin in Obesity-Associated Cognitive Decline and Alzheimer's Disease. Front. Neurosci. 12, 1027. https://doi.org/10.3389/fnins.2018.01027

Francis, H., Stevenson, R., 2013. The longer-term impacts of Western diet on human cognition and the brain. Appetite 63, 119–128. https://doi.org/10.1016/j.appet.2012.12.018

Frayn, K.N., 2000. Visceral fat and insulin resistance--causative or correlative? Br. J. Nutr. 83 Suppl 1, S71-7. https://doi.org/10.1017/s0007114500000982

Freedland, E.S., 2004. Role of a critical visceral adipose tissue threshold (CVATT) in metabolic syndrome: implications for controlling dietary carbohydrates: a review. Nutr. Metab. (Lond). 1, 12. https://doi.org/10.1186/1743-7075-1-12

Frühbeck, G., 2006. Intracellular signalling pathways activated by leptin. Biochem. J. 393, 7–20. https://doi.org/10.1042/BJ20051578

Furuya, S., Mitoma, J., Hirabayashi, Y., 2002. [Glia-derived L-Ser regulating survival development and lipid synthesis of developing central neurons]. Tanpakushitsu Kakusan Koso. 47, 409–416.

Furuya, S., Tabata, T., Mitoma, J., Yamada, K., Yamasaki, M., Makino, A., Yamamoto, T., Watanabe, M., Kano, M., Hirabayashi, Y., 2000. L-Serine and glycine serve as major astroglia-derived trophic factors for cerebellar Purkinje neurons. Proc. Natl. Acad. Sci. U. S. A. 97, 11528–11533. https://doi.org/10.1073/pnas.200364497

Galic, S., Oakhill, J.S., Steinberg, G.R., 2010. Adipose tissue as an endocrine organ. Mol. Cell. Endocrinol. 316, 129–139. https://doi.org/10.1016/j.mce.2009.08.018

Gammelsaeter, R., Coppola, T., Marcaggi, P., Storm-Mathisen, J., Chaudhry, F.A., Attwell, D., Regazzi, R., Gundersen, V., 2011. A role for glutamate transporters in the regulation of insulin secretion. PLoS One 6, e22960. https://doi.org/10.1371/journal.pone.0022960

Ge, Y., Dong, Z., Bagot, R.C., Howland, J.G., Phillips, A.G., Wong, T.P., Wang, Y.T., 2010. Hippocampal long-term depression is required for the consolidation of spatial memory. Proc. Natl. Acad. Sci. U. S. A. 107, 16697–16702. https://doi.org/10.1073/pnas.1008200107

González-Becerra, K., Ramos-Lopez, O., Barrón-Cabrera, E., Riezu-Boj, J.I., Milagro, F.I., Martínez-López, E., Martínez, J.A., 2019. Fatty acids, epigenetic mechanisms and chronic diseases: a systematic review. Lipids Health Dis. 18, 178. https://doi.org/10.1186/s12944-019-1120-6

Goossens, G.H., 2017. The Metabolic Phenotype in Obesity: Fat Mass, Body Fat Distribution, and Adipose Tissue Function. Obes. Facts 10, 207–215. https://doi.org/10.1159/000471488

Greenwood, C.E., Winocur, G., 1996. Cognitive impairment in rats fed high-fat diets: a specific effect of saturated fatty-acid intake. Behav. Neurosci. 110, 451–459. https://doi.org/10.1037//0735-7044.110.3.451

Grigoryan, G., Korkotian, E., Segal, M., 2012. Selective facilitation of LTP in the ventral hippocampus by calcium stores. Hippocampus 22, 1635–1644. https://doi.org/10.1002/hipo.22000

Gruzdeva, O., Borodkina, D., Uchasova, E., Dyleva, Y., Barbarash, O., 2019. Leptin resistance: underlying mechanisms and diagnosis. Diabetes. Metab. Syndr. Obes. 12, 191–198. https://doi.org/10.2147/DMSO.S182406

Gupta, R.K., Arany, Z., Seale, P., Mepani, R.J., Ye, L., Conroe, H.M., Roby, Y.A., Kulaga, H., Reed, R.R., Spiegelman, B.M., 2010. Transcriptional control of preadipocyte determination by Zfp423. Nature 464, 619–623. https://doi.org/10.1038/nature08816

Gupta, Sunita, Knight, A.G., Gupta, Shruti, Keller, J.N., Bruce-Keller, A.J., 2012. Saturated long-chain fatty acids activate inflammatory signaling in astrocytes. J. Neurochem. 120, 1060–1071. https://doi.org/10.1111/j.1471-4159.2012.07660.x

Guzmán-Ruiz, R., Stucchi, P., Ramos, M.P., Sevillano, J., Somoza, B., Fernández-Alfonso, M., Ruiz-Gayo, M., 2012. Leptin drives fat distribution during diet-induced obesity in mice. Endocrinol. y Nutr. organo la Soc. Esp. Endocrinol. y Nutr. 59, 354–361. https://doi.org/10.1016/j.endonu.2012.04.001

Habinowski, S.A., Witters, L.A., 2001. The effects of AICAR on adipocyte differentiation of 3T3-L1 cells. Biochem. Biophys. Res. Commun. 286, 852–856. https://doi.org/10.1006/bbrc.2001.5484

Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., Friedman, J.M., 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269, 543–546. https://doi.org/10.1126/science.7624777

Hamad, M.I.K., Ma-Högemeier, Z.-L., Riedel, C., Conrads, C., Veitinger, T., Habijan, T., Schulz, J.-N., Krause, M., Wirth, M.J., Hollmann, M., Wahle, P., 2011. Cell class-specific regulation of neocortical dendrite and spine growth by AMPA receptor splice and editing variants. Development 138, 4301–4313. https://doi.org/10.1242/dev.071076

Hamilton, M.T., Hamilton, D.G., Zderic, T.W., 2007. Role of low energy expenditure and sitting in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. Diabetes 56, 2655–2667. https://doi.org/10.2337/db07-0882

Han, H.J., Park, S.H., 2011. High glucose stimulates glutamate uptakes in pancreatic β-cells. Lab. Anim. Res. 27, 327–331. https://doi.org/10.5625/lar.2011.27.4.327

Han, J.C., Lawlor, D.A., Kimm, S.Y.S., 2010. Childhood obesity. Lancet (London, England) 375, 1737–1748. https://doi.org/10.1016/S0140-6736(10)60171-7

Hao, S., Dey, A., Yu, X., Stranahan, A.M., 2016. Dietary obesity reversibly induces synaptic stripping by microglia and impairs hippocampal plasticity. Brain. Behav. Immun. 51, 230–239. https://doi.org/10.1016/j.bbi.2015.08.023

Hardingham, G.E., Fukunaga, Y., Bading, H., 2002. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nat. Neurosci. 5, 405–414. https://doi.org/10.1038/nn835

Harris, A.Z., Pettit, D.L., 2008. Recruiting extrasynaptic NMDA receptors augments synaptic signaling. J. Neurophysiol. 99, 524–533. https://doi.org/10.1152/jn.01169.2007

Harris, R.B.S., 2014. Direct and indirect effects of leptin on adipocyte metabolism. Biochim. Biophys. Acta 1842, 414–423. https://doi.org/10.1016/j.bbadis.2013.05.009

Harrold, J.A., Cai, X., Williams, G., 1998. Leptin, the hypothalamus and the regulation of

adiposity. Curr. Opin. Lipidol. 9, 295–299. https://doi.org/10.1097/00041433-199808000-00002

Harvey, J., 2007a. Leptin: a diverse regulator of neuronal function. J. Neurochem. 100, 307–313. https://doi.org/10.1111/j.1471-4159.2006.04205.x

Harvey, J., 2007b. Leptin regulation of neuronal excitability and cognitive function. Curr. Opin. Pharmacol. 7, 643–647. https://doi.org/10.1016/j.coph.2007.10.006

Havel, P.J., 2001. Peripheral signals conveying metabolic information to the brain: short-term and long-term regulation of food intake and energy homeostasis. Exp. Biol. Med. (Maywood). 226, 963–977. https://doi.org/10.1177/153537020122601102

Heaney, M.L., Golde, D.W., 1993. Soluble hormone receptors. Blood.

Hellmuth, C., Demmelmair, H., Schmitt, I., Peissner, W., Blüher, M., Koletzko, B., 2013. Association between plasma nonesterified fatty acids species and adipose tissue fatty acid composition. PLoS One 8, e74927. https://doi.org/10.1371/journal.pone.0074927

Henneberger, C., Papouin, T., Oliet, S.H.R., Rusakov, D.A., 2010. Long-term potentiation depends on release of d-serine from astrocytes. Nature 463, 232–236. https://doi.org/10.1038/nature08673

Herring, B.E., Nicoll, R.A., 2016. Long-Term Potentiation: From CaMKII to AMPA Receptor Trafficking. Annu. Rev. Physiol. 78, 351–365. https://doi.org/10.1146/annurev-physiol-021014-071753

Heshka, J.T., Jones, P.J., 2001. A role for dietary fat in leptin receptor, OB-Rb, function. Life Sci. 69, 987–1003. https://doi.org/10.1016/s0024-3205(01)01201-2

Hirabayashi, Y., Furuya, S., 2008. Roles of l-serine and sphingolipid synthesis in brain development and neuronal survival. Prog. Lipid Res. 47, 188–203. https://doi.org/10.1016/j.plipres.2008.01.003

Hoane, M.R., Swan, A.A., Heck, S.E., 2011. The effects of a high-fat sucrose diet on functional outcome following cortical contusion injury in the rat. Behav. Brain Res. 223, 119–124. https://doi.org/10.1016/j.bbr.2011.04.028

Hsieh, C.-M., Liu, P.Y., Chin, L.K., Zhang, J.B., Wang, K., Sung, K.-B., Ser, W., Bourouina, T., Leprince-Wang, Y., Liu, A.-Q., 2019. Regulation of lipid droplets in live preadipocytes using optical diffraction tomography and Raman spectroscopy. Opt. Express 27, 22994–23008. https://doi.org/10.1364/OE.27.022994

Hsuchou, H., He, Y., Kastin, A.J., Tu, H., Markadakis, E.N., Rogers, R.C., Fossier, P.B., Pan, W., 2009. Obesity induces functional astrocytic leptin receptors in hypothalamus. Brain 132, 889–902. https://doi.org/10.1093/brain/awp029

Huan, J.-N., Li, J., Han, Y., Chen, K., Wu, N., Zhao, A.Z., 2003. Adipocyte-selective reduction of the leptin receptors induced by antisense RNA leads to increased adiposity, dyslipidemia, and insulin resistance. J. Biol. Chem. 278, 45638–45650. https://doi.org/10.1074/jbc.M304165200

Huang, H., Song, T.-J., Li, X., Hu, L., He, Q., Liu, M., Lane, M.D., Tang, Q.-Q., 2009. BMP signaling pathway is required for commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. Proc. Natl. Acad. Sci. U. S. A. 106, 12670–12675. https://doi.org/10.1073/pnas.0906266106

Huszar, D., Lynch, C.A., Fairchild-Huntress, V., Dunmore, J.H., Fang, Q., Berkemeier, L.R., Gu, W., Kesterson, R.A., Boston, B.A., Cone, R.D., Smith, F.J., Campfield, L.A., Burn, P., Lee, F., 1997. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88, 131–141. https://doi.org/10.1016/s0092-8674(00)81865-6

Hwang, L.L., Wang, C.H., Li, T.L., Chang, S.D., Lin, L.C., Chen, C.P., Chen, C.T., Liang, K.C., Ho, I.K., Yang, W.S., Chiou, L.C., 2010. Sex differences in high-fat diet-induced obesity, metabolic alterations and learning, and synaptic plasticity deficits in mice. Obesity 18, 463–469. https://doi.org/10.1038/oby.2009.273

Ibrahim, M.M., 2010. Subcutaneous and visceral adipose tissue: Structural and functional differences. Obes. Rev. 11, 11–18. https://doi.org/10.1111/j.1467-789X.2009.00623.x

Imamura, F., O'Connor, L., Ye, Z., Mursu, J., Hayashino, Y., Bhupathiraju, S.N., Forouhi, N.G., 2015. Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: systematic review, meta-analysis, and estimation of population attributable fraction. BMJ 351, h3576. https://doi.org/10.1136/bmj.h3576

Izawa, S., Yoneshiro, T., Kondoh, K., Nakagiri, S., Okamatsu-Ogura, Y., Terao, A., Minokoshi, Y., Yamanaka, A., Kimura, K., 2022. Melanin-concentrating hormone-producing neurons in the hypothalamus regulate brown adipose tissue and thus contribute to energy expenditure. J. Physiol. 600, 815–827. https://doi.org/10.1113/JP281241

Izquierdo, A.G., Crujeiras, A.B., Casanueva, F.F., Carreira, M.C., 2019. Leptin, Obesity, and Leptin Resistance: Where Are We 25 Years Later? Nutrients 11. https://doi.org/10.3390/nu11112704

Jagannathan, L., Socks, E., Balasubramanian, P., McGowan, R., Herdt, T.M., Kianian, R., MohanKumar, S.M.J., MohanKumar, P.S., 2020. Oleic acid stimulates monoamine efflux through PPAR-α: Differential effects in diet-induced obesity. Life Sci. 255, 117867. https://doi.org/10.1016/j.lfs.2020.117867

Jensen, N.J., Wodschow, H.Z., Nilsson, M., Rungby, J., 2020. Effects of Ketone Bodies on Brain Metabolism and Function in Neurodegenerative Diseases. Int. J. Mol. Sci. 21.

https://doi.org/10.3390/ijms21228767

Kaczmarczyk, M.M., Machaj, A.S., Chiu, G.S., Lawson, M.A., Gainey, S.J., York, J.M., Meling, D.D., Martin, S.A., Kwakwa, K.A., Newman, A.F., Woods, J.A., Kelley, K.W., Wang, Y., Miller, M.J., Freund, G.G., 2013. Methylphenidate prevents high-fat diet (HFD)-induced learning/memory impairment in juvenile mice. Psychoneuroendocrinology 38, 1553–1564. https://doi.org/10.1016/j.psyneuen.2013.01.004

Kakuma, T., Wang, Z.W., Pan, W., Unger, R.H., Zhou, Y.T., 2000. Role of leptin in peroxisome proliferator-activated receptor gamma coactivator-1 expression. Endocrinology 141, 4576–4582. https://doi.org/10.1210/endo.141.12.7804

Kalra, S.P., Dube, M.G., Pu, S., Xu, B., Horvath, T.L., Kalra, P.S., 1999. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr. Rev. 20, 68–100. https://doi.org/10.1210/edrv.20.1.0357

Kandel, E.R., 2001. The molecular biology of memory storage: a dialogue between genes and synapses. Science 294, 1030–1038. https://doi.org/10.1126/science.1067020

Kandimalla, R., Thirumala, V., Reddy, P.H., 2017. Is Alzheimer's disease a Type 3 Diabetes? A critical appraisal. Biochim. Biophys. acta. Mol. basis Dis. 1863, 1078–1089. https://doi.org/10.1016/j.bbadis.2016.08.018

Kanoski, S.E., Meisel, R.L., Mullins, A.J., Davidson, T.L., 2007. The effects of energy-rich diets on discrimination reversal learning and on BDNF in the hippocampus and prefrontal cortex of the rat. Behav. Brain Res. 182, 57–66. https://doi.org/10.1016/j.bbr.2007.05.004

Kaplan, R.J., Greenwood, C.E., 1998. Dietary saturated fatty acids and brain function. Neurochem. Res. 23, 615–626. https://doi.org/10.1023/a:1022478503367

Kennedy, A., Martinez, K., Chuang, C.-C., LaPoint, K., McIntosh, M., 2009. Saturated fatty acid-mediated inflammation and insulin resistance in adipose tissue: mechanisms of action and implications. J. Nutr. 139, 1–4. https://doi.org/10.3945/jn.108.098269

Keung, W., Palaniyappan, A., Lopaschuk, G.D., 2011. Chronic central leptin decreases food intake and improves glucose tolerance in diet-induced obese mice independent of hypothalamic malonyl CoA levels and skeletal muscle insulin sensitivity. Endocrinology 152, 4127–4137. https://doi.org/10.1210/en.2011-1254

Kim, J.H., Choi, J.-H., 2013. Pathophysiology and clinical characteristics of hypothalamic obesity in children and adolescents. Ann. Pediatr. Endocrinol. Metab. 18, 161–167. https://doi.org/10.6065/apem.2013.18.4.161

Knothe, G., Dunn, R.O., 2009. A Comprehensive Evaluation of the Melting Points of Fatty Acids and Esters Determined by Differential Scanning Calorimetry. J. Am. Oil Chem. Soc. 86, 843–856. https://doi.org/https://doi.org/10.1007/s11746-009-1423-2

Kobayashi, K., 2010. Hippocampal mossy fiber synaptic transmission and its modulation. Vitam. Horm. 82, 65–85. https://doi.org/10.1016/S0083-6729(10)82004-7

Koch, C.E., Lowe, C., Pretz, D., Steger, J., Williams, L.M., Tups, A., 2014. High-fat diet induces leptin resistance in leptin-deficient mice. J. Neuroendocrinol. 26, 58–67. https://doi.org/10.1111/jne.12131

Kochlamazashvili, G., Senkov, O., Grebenyuk, S., Robinson, C., Xiao, M.-F., Stummeyer, K., Gerardy-Schahn, R., Engel, A.K., Feig, L., Semyanov, A., Suppiramaniam, V., Schachner, M., Dityatev, A., 2010. Neural cell adhesion molecule-associated polysialic acid regulates synaptic plasticity and learning by restraining the signaling through GluN2B-containing NMDA receptors. J. Neurosci. 30, 4171–4183. https://doi.org/10.1523/JNEUROSCI.5806-09.2010

Kopp, W., 2019. How western diet and lifestyle drive the pandemic of obesity and civilization diseases. Diabetes, Metab. Syndr. Obes. Targets Ther. 12, 2221–2236. https://doi.org/10.2147/DMSO.S216791

Kreier, F., Fliers, E., Voshol, P.J., Van Eden, C.G., Havekes, L.M., Kalsbeek, A., Van Heijningen, C.L., Sluiter, A.A., Mettenleiter, T.C., Romijn, J.A., Sauerwein, H.P., Buijs, R.M., 2002. Selective parasympathetic innervation of subcutaneous and intra-abdominal fat--functional implications. J. Clin. Invest. 110, 1243–1250. https://doi.org/10.1172/JCI15736

Kutoh, E., Boss, O., Levasseur, F., Giacobino, J.P., 1998. Quantification of the full length leptin receptor (OB-Rb) in human brown and white adipose tissue. Life Sci. 62, 445–451. https://doi.org/10.1016/s0024-3205(97)01138-7

Labban, R.S.M., Alfawaz, H., Almnaizel, A.T., Hassan, W.M., 2020. High - fat diet - induced obesity and impairment of brain neurotransmitter pool 147–160.

Lathe, R., 2001. Hormones and the hippocampus. J. Endocrinol. 169, 205–231. https://doi.org/10.1677/joe.0.1690205

Ledoux, S., Queguiner, I., Msika, S., Calderari, S., Rufat, P., Gasc, J.-M., Corvol, P., Larger, E., 2008. Angiogenesis associated with visceral and subcutaneous adipose tissue in severe human obesity. Diabetes 57, 3247–3257. https://doi.org/10.2337/db07-1812

Lee, J.C., Greig, A., Ravindranathan, A., Parks, T.N., Rao, M.S., 1998. Molecular analysis of AMPA-specific receptors: subunit composition, editing, and calcium influx determination in small amounts of tissue. Brain Res. Brain Res. Protoc. 3, 142–154. https://doi.org/10.1016/s1385-299x(98)00035-x

Lee, Y., Yu, X., Gonzales, F., Mangelsdorf, D.J., Wang, M.-Y., Richardson, C., Witters, L.A., Unger, R.H., 2002. PPAR alpha is necessary for the lipopenic action of hyperleptinemia on white adipose and liver tissue. Proc. Natl. Acad. Sci. U. S. A. 99, 11848–11853.

https://doi.org/10.1073/pnas.182420899

Leranth, C., Hajszan, T., 2007. Extrinsic afferent systems to the dentate gyrus. Prog. Brain Res. 163, 63–84. https://doi.org/10.1016/S0079-6123(07)63004-0

Li, X.L., Aou, S., Oomura, Y., Hori, N., Fukunaga, K., Hori, T., 2002. Impairment of long-term potentiation and spatial memory in leptin receptor-deficient rodents. Neuroscience 113, 607–615. https://doi.org/10.1016/S0306-4522(02)00162-8

Lim, S.S., Noakes, M., Keogh, J.B., Clifton, P.M., 2010. Long-term effects of a low carbohydrate, low fat or high unsaturated fat diet compared to a no-intervention control. Nutr. Metab. Cardiovasc. Dis. 20, 599–607. https://doi.org/10.1016/j.numecd.2009.05.003

Lisman, J., Schulman, H., Cline, H., 2002. The molecular basis of CaMKII function in synaptic and behavioural memory. Nat. Rev. Neurosci. 3, 175–190. https://doi.org/10.1038/nrn753

Liu, Y.C., Wang, H.-S., 2013. Medium-chain triglyceride ketogenic diet, an effective treatment for drug-resistant epilepsy and a comparison with other ketogenic diets. Biomed. J. 36, 9–15. https://doi.org/10.4103/2319-4170.107154

Lizarbe, B., Soares, A.F., Larsson, S., Duarte, J.M.N., 2019. Neurochemical modifications in the hippocampus, cortex and hypothalamus of mice exposed to long-term high-fat diet. Front. Neurosci. 13, 1–15. https://doi.org/10.3389/fnins.2018.00985

Ludwig, D.S., Mountjoy, K.G., Tatro, J.B., Gillette, J.A., Frederich, R.C., Flier, J.S., Maratos-Flier, E., 1998. Melanin-concentrating hormone: a functional melanocortin antagonist in the hypothalamus. Am. J. Physiol. 274, E627-33. https://doi.org/10.1152/ajpendo.1998.274.4.E627

Lüscher, C., Huber, K.M., 2010. Group 1 mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. Neuron 65, 445–459. https://doi.org/10.1016/j.neuron.2010.01.016

Lüscher, C., Malenka, R.C., 2012. NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). Cold Spring Harb. Perspect. Biol. 4. https://doi.org/10.1101/cshperspect.a005710

Lüscher, C., Xia, H., Beattie, E.C., Carroll, R.C., von Zastrow, M., Malenka, R.C., Nicoll, R.A., 1999. Role of AMPA receptor cycling in synaptic transmission and plasticity. Neuron 24, 649–658. https://doi.org/10.1016/s0896-6273(00)81119-8

Machinal-Quélin, F., Dieudonné, M.N., Leneveu, M.C., Pecquery, R., Giudicelli, Y., 2002. Proadipogenic effect of leptin on rat preadipocytes in vitro: activation of MAPK and STAT3 signaling pathways. Am. J. Physiol. Cell Physiol. 282, C853-63. https://doi.org/10.1152/ajpcell.00331.2001

Malenka, R.C., 1994. Synaptic plasticity in the hippocampus: LTP and LTD. Cell 78, 535–538.

https://doi.org/10.1016/0092-8674(94)90517-7

Malenka, R.C., Bear, M.F., 2004. LTP and LTD: an embarrassment of riches. Neuron 44, 5–21. https://doi.org/10.1016/j.neuron.2004.09.012

Malenka, R.C., Nicoll, R.A., 1999. Long-term potentiation--a decade of progress? Science 285, 1870–1874. https://doi.org/10.1126/science.285.5435.1870

Malinow, R., Malenka, R.C., 2002. AMPA receptor trafficking and synaptic plasticity. Annu. Rev. Neurosci. 25, 103–126. https://doi.org/10.1146/annurev.neuro.25.112701.142758

Manning, B.D., Toker, A., 2017. AKT/PKB Signaling: Navigating the Network. Cell 169, 381–405. https://doi.org/10.1016/j.cell.2017.04.001

Marcelin, G., Gautier, E.L., Clément, K., 2022. Adipose Tissue Fibrosis in Obesity: Etiology and Challenges. Annu. Rev. Physiol. 84, 135–155. https://doi.org/10.1146/annurev-physiol-060721-092930

Marette, A., 2003. Molecular mechanisms of inflammation in obesity-linked insulin resistance. Int. J. Obes. Relat. Metab. Disord. J. Int. Assoc. Study Obes. 27 Suppl 3, S46-8. https://doi.org/10.1038/sj.ijo.0802500

Matta, J.A., Ashby, M.C., Sanz-Clemente, A., Roche, K.W., Isaac, J.T.R., 2011. mGluR5 and NMDA receptors drive the experience- and activity-dependent NMDA receptor NR2B to NR2A subunit switch. Neuron 70, 339–351. https://doi.org/10.1016/j.neuron.2011.02.045

Matura, S., Prvulovic, D., Mohadjer, N., Fusser, F., Oertel, V., Reif, A., Pantel, J., Karakaya, T., 2021. Association of dietary fat composition with cognitive performance and brain morphology in cognitively healthy individuals. Acta Neuropsychiatr. 33, 134–140. https://doi.org/10.1017/neu.2021.1

Maugard, M., Vigneron, P.A., Bolaños, J.P., Bonvento, G., 2021. L-Serine links metabolism with neurotransmission. Prog. Neurobiol. 197. https://doi.org/10.1016/j.pneurobio.2020.101896

McCarty, M.F., DiNicolantonio, J.J., 2016. Lauric acid-rich medium-chain triglycerides can substitute for other oils in cooking applications and may have limited pathogenicity. Open Hear. 3, e000467. https://doi.org/10.1136/openhrt-2016-000467

McGregor, G., Harvey, J., 2018. Regulation of hippocampal synaptic function by the metabolic hormone, Leptin: Implications for health and neurodegenerative disease. Front. Cell. Neurosci. 12, 1–11. https://doi.org/10.3389/fncel.2018.00340

Mikkelsen, B., Williams, J., Rakovac, I., Wickramasinghe, K., Hennis, A., Shin, H.-R., Farmer, M., Weber, M., Berdzuli, N., Borges, C., Huber, M., Breda, J., 2019. Life course approach to prevention and control of non-communicable diseases. BMJ 364, l257. https://doi.org/10.1136/bmj.l257

Minokoshi, Y., Alquier, T., Furukawa, N., Kim, Y.-B., Lee, A., Xue, B., Mu, J., Foufelle, F., Ferré, P., Birnbaum, M.J., Stuck, B.J., Kahn, B.B., 2004. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature 428, 569–574. https://doi.org/10.1038/nature02440

Minokoshi, Y., Kim, Y.-B., Peroni, O.D., Fryer, L.G.D., Müller, C., Carling, D., Kahn, B.B., 2002. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 415, 339–343. https://doi.org/10.1038/415339a

Mizuno, T.M., Mobbs, C. V, 1999. Hypothalamic agouti-related protein messenger ribonucleic acid is inhibited by leptin and stimulated by fasting. Endocrinology 140, 814–817. https://doi.org/10.1210/endo.140.2.6491

Morris, M.C., Tangney, C.C., 2014. Dietary fat composition and dementia risk. Neurobiol. Aging 35 Suppl 2, S59-64. https://doi.org/10.1016/j.neurobiolaging.2014.03.038

Morrison, C.D., 2009. Leptin signaling in brain: A link between nutrition and cognition? Biochim. Biophys. Acta 1792, 401–408. https://doi.org/10.1016/j.bbadis.2008.12.004

Moult, P.R., Harvey, J., 2009. Regulation of glutamate receptor trafficking by leptin. Biochem. Soc. Trans. 37, 1364–1368. https://doi.org/10.1042/BST0371364

Müller, G., Ertl, J., Gerl, M., Preibisch, G., 1997. Leptin impairs metabolic actions of insulin in isolated rat adipocytes. J. Biol. Chem. 272, 10585–10593. https://doi.org/10.1074/jbc.272.16.10585

Muñoz-Garach, A., Cornejo-Pareja, I., Tinahones, F.J., 2016. Does Metabolically Healthy Obesity Exist? Nutrients 8. https://doi.org/10.3390/nu8060320

Münzberg, H., Flier, J.S., Bjørbæk, C., 2004. Region-Specific Leptin Resistance within the Hypothalamus of Diet-Induced Obese Mice. Endocrinology 145, 4880–4889. https://doi.org/10.1210/en.2004-0726

Murakami, G., Tsurugizawa, T., Hatanaka, Y., Komatsuzaki, Y., Tanabe, N., Mukai, H., Hojo, Y., Kominami, S., Yamazaki, T., Kimoto, T., Kawato, S., 2006. Comparison between basal and apical dendritic spines in estrogen-induced rapid spinogenesis of CA1 principal neurons in the adult hippocampus. Biochem. Biophys. Res. Commun. 351, 553–558. https://doi.org/10.1016/j.bbrc.2006.10.066

Myers, M.G., Cowley, M.A., Münzberg, H., 2008. Mechanisms of leptin action and leptin resistance. Annu. Rev. Physiol. 70, 537–556. https://doi.org/10.1146/annurev.physiol.70.113006.100707

Myers, M.G.J., Leibel, R.L., Seeley, R.J., Schwartz, M.W., 2010. Obesity and leptin resistance: distinguishing cause from effect. Trends Endocrinol. Metab. 21, 643–651. https://doi.org/10.1016/j.tem.2010.08.002

Naranjo, V., Contreras, A., Merino, B., Plaza, A., Lorenzo, M.P., García-Cáceres, C., García, A., Chowen, J.A., Ruiz-Gayo, M., Del Olmo, N., Cano, V., 2019. Specific Deletion of the Astrocyte Leptin Receptor Induces Changes in Hippocampus Glutamate Metabolism, Synaptic Transmission and Plasticity. Neuroscience. https://doi.org/10.1016/j.neuroscience.2019.10.005

Neame, S., Safory, H., Radzishevsky, I., Touitou, A., Marchesani, F., Marchetti, M., Kellner, S., Berlin, S., Foltyn, V.N., Engelender, S., Billard, J.M., Wolosker, H., 2019. The NMDA receptor activation by D-serine and glycine is controlled by an astrocytic Phgdh-dependent serine shuttle. Proc. Natl. Acad. Sci. U. S. A. 116, 20736–20742. https://doi.org/10.1073/pnas.1909458116

Nicolas, C.S., Peineau, S., Amici, M., Csaba, Z., Fafouri, A., Javalet, C., Collett, V.J., Hildebrandt, L., Seaton, G., Choi, S.-L., Sim, S.-E., Bradley, C., Lee, K., Zhuo, M., Kaang, B.-K., Gressens, P., Dournaud, P., Fitzjohn, S.M., Bortolotto, Z.A., Cho, K., Collingridge, G.L., 2012. The Jak/STAT pathway is involved in synaptic plasticity. Neuron 73, 374–390. https://doi.org/10.1016/j.neuron.2011.11.024

Nilsson, L.-G., Nilsson, E., 2009. Overweight and cognition. Scand. J. Psychol. 50, 660–667. https://doi.org/10.1111/j.1467-9450.2009.00777.x

Nonaka, Y., Takagi, T., Inai, M., Nishimura, S., Urashima, S., Honda, K., Aoyama, T., Terada, S., 2016. Lauric acid stimulates ketone body production in the KT-5 astrocyte cell line. J. Oleo Sci. 65, 693–699. https://doi.org/10.5650/jos.ess16069

O'Malley, D., MacDonald, N., Mizielinska, S., Connolly, C.N., Irving, A.J., Harvey, J., 2007. Leptin promotes rapid dynamic changes in hippocampal dendritic morphology. Mol. Cell. Neurosci. 35, 559–572. https://doi.org/10.1016/j.mcn.2007.05.001

Oh, H., Boghossian, S., York, D.A., Park-York, M., 2013. The effect of high fat diet and saturated fatty acids on insulin signaling in the amygdala and hypothalamus of rats. Brain Res. 1537, 191–200. https://doi.org/10.1016/j.brainres.2013.09.025

Ollmann, M.M., Wilson, B.D., Yang, Y.K., Kerns, J.A., Chen, Y., Gantz, I., Barsh, G.S., 1997. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. Science 278, 135–138. https://doi.org/10.1126/science.278.5335.135

Oomura, Y., Hori, N., Shiraishi, T., Fukunaga, K., Takeda, H., Tsuji, M., Matsumiya, T., Ishibashi, M., Aou, S., Li, X.L., Kohno, D., Uramura, K., Sougawa, H., Yada, T., Wayner, M.J., Sasaki, K., 2006. Leptin facilitates learning and memory performance and enhances hippocampal CA1 long-term potentiation and CaMK II phosphorylation in rats. Peptides 27, 2738–2749. https://doi.org/10.1016/j.peptides.2006.07.001

Ota, M., Matsuo, J., Ishida, I., Takano, H., Yokoi, Y., Hori, H., Yoshida, S., Ashida, K., Nakamura, K., Takahashi, T., Kunugi, H., 2019. Effects of a medium-chain triglyceride-

based ketogenic formula on cognitive function in patients with mild-to-moderate Alzheimer's disease. Neurosci. Lett. 690, 232–236. https://doi.org/10.1016/j.neulet.2018.10.048

Panatier, A., Theodosis, D.T., Mothet, J.P., Touquet, B., Pollegioni, L., Poulain, D.A., Oliet, S.H.R., 2006. Glia-Derived d-Serine Controls NMDA Receptor Activity and Synaptic Memory. Cell 125, 775–784. https://doi.org/10.1016/j.cell.2006.02.051

Park, H.R., Kim, J.-Y., Park, K.-Y., Lee, J., 2011. Lipotoxicity of palmitic Acid on neural progenitor cells and hippocampal neurogenesis. Toxicol. Res. 27, 103–110. https://doi.org/10.5487/TR.2011.27.2.103

Patil, S., Balu, D., Melrose, J., Chan, C., 2008. Brain region-specificity of palmitic acid-induced abnormalities associated with Alzheimer's disease. BMC Res. Notes 1, 20. https://doi.org/10.1186/1756-0500-1-20

Patil, S., Melrose, J., Chan, C., 2007. Involvement of astroglial ceramide in palmitic acid-induced Alzheimer-like changes in primary neurons. Eur. J. Neurosci. 26, 2131–2141. https://doi.org/10.1111/j.1460-9568.2007.05797.x

Patil, S., Sheng, L., Masserang, A., Chan, C., 2006. Palmitic acid-treated astrocytes induce BACE1 upregulation and accumulation of C-terminal fragment of APP in primary cortical neurons. Neurosci. Lett. 406, 55–59. https://doi.org/10.1016/j.neulet.2006.07.015

Pavlowsky, A., Alarcon, J.M., 2012. Interaction between long-term potentiation and depression in CA1 synapses: temporal constrains, functional compartmentalization and protein synthesis. PLoS One 7, e29865. https://doi.org/10.1371/journal.pone.0029865

Peineau, S., Taghibiglou, C., Bradley, C., Wong, T.P., Liu, L., Lu, J., Lo, E., Wu, D., Saule, E., Bouschet, T., Matthews, P., Isaac, J.T.R., Bortolotto, Z.A., Wang, Y.T., Collingridge, G.L., 2007. LTP inhibits LTD in the hippocampus via regulation of GSK3beta. Neuron 53, 703–717. https://doi.org/10.1016/j.neuron.2007.01.029

Phillips, L.J., McGorry, P.D., Garner, B., Thompson, K.N., Pantelis, C., Wood, S.J., Berger, G., 2006. Stress, the hippocampus and the hypothalamic-pituitary-adrenal axis: implications for the development of psychotic disorders. Aust. N. Z. J. Psychiatry 40, 725–741. https://doi.org/10.1080/j.1440-1614.2006.01877.x

Phillips, R., Ursell, T., Wiggins, P., Sens, P., 2009. Emerging roles for lipids in shaping membrane-protein function. Nature 459, 379–385. https://doi.org/10.1038/nature08147

Pitt, D., Werner, P., Raine, C.S., 2000. Glutamate excitotoxicity in a model of multiple sclerosis. Nat. Med. 6, 67–70. https://doi.org/10.1038/71555

Plaza, A., Antonazzi, M., Blanco-Urgoiti, J., Del Olmo, N., Ruiz-Gayo, M., 2019a. Potential Role of Leptin in Cardiac Steatosis Induced by Highly Saturated Fat Intake during Adolescence.

Mol. Nutr. Food Res. 63. https://doi.org/10.1002/mnfr.201900110

Plaza, A., Antonazzi, M., Blanco-Urgoiti, J., Del Olmo, N., Ruiz-Gayo, M., 2019b. Potential Role of Leptin in Cardiac Steatosis Induced by Highly Saturated Fat Intake during Adolescence. Mol. Nutr. Food Res. 63, 1–11. https://doi.org/10.1002/mnfr.201900110

Plaza, A., Antonazzi, M., Blanco-Urgoiti, J., Del Olmo, N., Ruiz-Gayo, M., 2019c. Potential Role of Leptin in Cardiac Steatosis Induced by Highly Saturated Fat Intake during Adolescence. Mol. Nutr. Food Res. 63, e1900110. https://doi.org/10.1002/mnfr.201900110

Pochini, L., Scalise, M., Galluccio, M., Indiveri, C., 2014. Membrane transporters for the special amino acid glutamine: structure/function relationships and relevance to human health. Front. Chem. 2, 61. https://doi.org/10.3389/fchem.2014.00061

Pou, K.M., Massaro, J.M., Hoffmann, U., Vasan, R.S., Maurovich-Horvat, P., Larson, M.G., Keaney, J.F.J., Meigs, J.B., Lipinska, I., Kathiresan, S., Murabito, J.M., O'Donnell, C.J., Benjamin, E.J., Fox, C.S., 2007. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. Circulation 116, 1234–1241. https://doi.org/10.1161/CIRCULATIONAHA.107.710509

Preston, A.R., Eichenbaum, H., 2013. Interplay of hippocampus and prefrontal cortex in memory. Curr. Biol. 23, R764-73. https://doi.org/10.1016/j.cub.2013.05.041

Qu, D., Ludwig, D.S., Gammeltoft, S., Piper, M., Pelleymounter, M.A., Cullen, M.J., Mathes, W.F., Przypek, R., Kanarek, R., Maratos-Flier, E., 1996. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. Nature 380, 243–247. https://doi.org/10.1038/380243a0

Ramsay, T.G., 2005. Porcine preadipocyte proliferation and differentiation: a role for leptin? J. Anim. Sci. 83, 2066–2074. https://doi.org/10.2527/2005.8392066x

Ramsay, T.G., Richards, M.P., 2004. Hormonal regulation of leptin and leptin receptor expression in porcine subcutaneous adipose tissue. J. Anim. Sci. 82, 3486–3492. https://doi.org/10.2527/2004.82123486x

Ravaut, G., Légiot, A., Bergeron, K.-F., Mounier, C., 2020. Monounsaturated Fatty Acids in Obesity-Related Inflammation. Int. J. Mol. Sci. 22. https://doi.org/10.3390/ijms22010330

Ribeiro, M., Castelhano, J., Petrella, L.I., Sereno, J., Rodrigues, T., Neves, C., Letra, L., Baptista, F.I., Seiça, R., Matafome, P., Castelo-Branco, M., 2018. High-fat diet induces a neurometabolic state characterized by changes in glutamate and N-acetylaspartate pools associated with early glucose intolerance: An in vivo multimodal MRI study. J. Magn. Reson. Imaging 48, 757–766. https://doi.org/10.1002/jmri.25942

Richard, M., 2006. 581Theories of Hippocampal Function. Hippocampus B.

https://doi.org/10.1093/acprof:oso/9780195100273.003.0013

Roger, C., Lasbleiz, A., Guye, M., Dutour, A., Gaborit, B., Ranjeva, J.-P., 2021. The Role of the Human Hypothalamus in Food Intake Networks: An MRI Perspective. Front. Nutr. 8, 760914. https://doi.org/10.3389/fnut.2021.760914

Ronquillo, M.D., Mellnyk, A., Cárdenas-Rodríguez, N., Martínez, E., Comoto, D.A., Carmona-Aparicio, L., Herrera, N.E., Lara, E., Pereyra, A., Floriano-Sánchez, E., 2019. Different gene expression profiles in subcutaneous & visceral adipose tissues from Mexican patients with obesity. Indian J. Med. Res. 149, 616–626. https://doi.org/10.4103/ijmr.IJMR_1165_17

Rosen, E.D., MacDougald, O.A., 2006. Adipocyte differentiation from the inside out. Nat. Rev. Mol. Cell Biol. 7, 885–896. https://doi.org/10.1038/nrm2066

Rothstein, J.D., Van Kammen, M., Levey, A.I., Martin, L.J., Kuncl, R.W., 1995. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann. Neurol. 38, 73–84. https://doi.org/10.1002/ana.410380114

Sáinz, N., Rodríguez, A., Catalán, V., Becerril, S., Ramírez, B., Gómez-Ambrosi, J., Frühbeck, G., 2009. Leptin administration favors muscle mass accretion by decreasing FoxO3a and increasing PGC-1alpha in ob/ob mice. PLoS One 4, e6808. https://doi.org/10.1371/journal.pone.0006808

Sales-Campos, H., Souza, P.R. de, Peghini, B.C., da Silva, J.S., Cardoso, C.R., 2013. An overview of the modulatory effects of oleic acid in health and disease. Mini Rev. Med. Chem. 13, 201–210.

Sans, N., Vissel, B., Petralia, R.S., Wang, Y.-X., Chang, K., Royle, G.A., Wang, C.-Y., O'Gorman, S., Heinemann, S.F., Wenthold, R.J., 2003. Aberrant formation of glutamate receptor complexes in hippocampal neurons of mice lacking the GluR2 AMPA receptor subunit. J. Neurosci. 23, 9367–9373. https://doi.org/10.1523/JNEUROSCI.23-28-09367.2003

Schaab, M., Kausch, H., Klammt, J., Nowicki, M., Anderegg, U., Gebhardt, R., Rose-John, S., Scheller, J., Thiery, J., Kratzsch, J., 2012. Novel regulatory mechanisms for generation of the soluble leptin receptor: implications for leptin action. PLoS One 7, e34787. https://doi.org/10.1371/journal.pone.0034787

Schulz, C., Paulus, K., Lehnert, H., 2010. Adipocyte-brain: crosstalk. Results Probl. Cell Differ. 52, 189–201. https://doi.org/10.1007/978-3-642-14426-4_16

Schwartz, M.W., Seeley, R.J., Campfield, L.A., Burn, P., Baskin, D.G., 1996. Identification of targets of leptin action in rat hypothalamus. J. Clin. Invest. 98, 1101–1106. https://doi.org/10.1172/JCI118891

Shanley, L.J., Irving, A.J., Harvey, J., 2001. Leptin enhances NMDA receptor function and modulates hippocampal synaptic plasticity. J. Neurosci. 21, 1–6. https://doi.org/10.1523/jneurosci.21-24-j0001.2001

Shimizu, H., Mori, M., 2005. The brain-adipose axis: a review of involvement of molecules. Nutr. Neurosci. 8, 7–20. https://doi.org/10.1080/10284150500047245

Shoham, N., Gefen, A., 2012. Mechanotransduction in adipocytes. J. Biomech. 45, 1–8. https://doi.org/10.1016/j.jbiomech.2011.10.023

Shoham, N., Girshovitz, P., Katzengold, R., Shaked, N.T., Benayahu, D., Gefen, A., 2014. Adipocyte stiffness increases with accumulation of lipid droplets. Biophys. J. 106, 1421–1431. https://doi.org/10.1016/j.bpj.2014.01.045

Siersbæk, R., Nielsen, R., Mandrup, S., 2012. Transcriptional networks and chromatin remodeling controlling adipogenesis. Trends Endocrinol. Metab. 23, 56–64. https://doi.org/10.1016/j.tem.2011.10.001

Simón, E., Del Barrio, A.S., 2002. [Leptin and obesity]. An. Sist. Sanit. Navar. 25 Suppl 1, 53–64. https://doi.org/10.23938/ASSN.0814

Simopoulos, A.P., 2016. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. Nutrients 8, 128. https://doi.org/10.3390/nu8030128

Smith, C.C., Vedder, L.C., McMahon, L.L., 2009. Estradiol and the relationship between dendritic spines, NR2B containing NMDA receptors, and the magnitude of long-term potentiation at hippocampal CA3-CA1 synapses. Psychoneuroendocrinology 34 Suppl 1, S130-42. https://doi.org/10.1016/j.psyneuen.2009.06.003

Soeda, S., Tsuji, Y., Ochiai, T., Mishima, K., Iwasaki, K., Fujiwara, M., Yokomatsu, T., Murano, T., Shibuya, S., Shimeno, H., 2004. Inhibition of sphingomyelinase activity helps to prevent neuron death caused by ischemic stress. Neurochem. Int. 45, 619–626. https://doi.org/10.1016/j.neuint.2004.04.001

Sohn, J.-W., 2015. Network of hypothalamic neurons that control appetite. BMB Rep. 48, 229–233. https://doi.org/10.5483/bmbrep.2015.48.4.272

Spoto, B., Di Betta, E., Mattace-Raso, F., Sijbrands, E., Vilardi, A., Parlongo, R.M., Pizzini, P., Pisano, A., Vermi, W., Testa, A., Cutrupi, S., D'Arrigo, G., Lonardi, S., Tripepi, G., Cancarini, G., Zoccali, C., 2014. Pro- and anti-inflammatory cytokine gene expression in subcutaneous and visceral fat in severe obesity. Nutr. Metab. Cardiovasc. Dis. 24, 1137–1143. https://doi.org/10.1016/j.numecd.2014.04.017

Stanley, B.G., Chin, A.S., Leibowitz, S.F., 1985. Feeding and drinking elicited by central injection of neuropeptide Y: evidence for a hypothalamic site(s) of action. Brain Res. Bull. 14, 521–524. https://doi.org/10.1016/0361-9230(85)90100-5

Strack, S., Colbran, R.J., 1998. Autophosphorylation-dependent targeting of calcium/ calmodulin-dependent protein kinase II by the NR2B subunit of the N-methyl- D-aspartate receptor. J. Biol. Chem. 273, 20689–20692. https://doi.org/10.1074/jbc.273.33.20689

Strack, S., McNeill, R.B., Colbran, R.J., 2000. Mechanism and regulation of calcium/calmodulin-dependent protein kinase II targeting to the NR2B subunit of the N-methyl-D-aspartate receptor. J. Biol. Chem. 275, 23798–23806. https://doi.org/10.1074/jbc.M001471200

Stranahan, A.M., Mattson, M.P., 2008. Impact of energy intake and expenditure on neuronal plasticity. Neuromolecular Med. 10, 209–218. https://doi.org/10.1007/s12017-008-8043-0

Stucchi, P., Guzmán-Ruiz, R., Gil-Ortega, M., Merino, B., Somoza, B., Cano, V., de Castro, J., Sevillano, J., Ramos, M.P., Fernández-Alfonso, M.S., Ruiz-Gayo, M., 2011. Leptin resistance develops spontaneously in mice during adult life in a tissue-specific manner. Consequences for hepatic steatosis. Biochimie 93, 1779–1785. https://doi.org/10.1016/j.biochi.2011.06.020

Swanger, S.A., Traynelis, S.F., 2018. Synaptic Receptor Diversity Revealed Across Space and Time. Trends Neurosci. 41, 486–488. https://doi.org/10.1016/j.tins.2018.06.001

Szirmai, I., Buzsáki, G., Kamondi, A., 2012. 120 years of hippocampal Schaffer collaterals. Hippocampus 22, 1508–1516. https://doi.org/10.1002/hipo.22001

Tabernero, A., Lavado, E.M., Granda, B., Velasco, A., Medina, J.M., 2001. Neuronal differentiation is triggered by oleic acid synthesized and released by astrocytes. J. Neurochem. 79, 606–616. https://doi.org/10.1046/j.1471-4159.2001.00598.x

Tamura, R., Eifuku, S., Uwano, T., Sugimori, M., Uchiyama, K., Ono, T., 2011. A method for recording evoked local field potentials in the primate dentate gyrus in vivo. Hippocampus 21, 565–574. https://doi.org/10.1002/hipo.20773

Tang, B.L., 2008. Leptin as a neuroprotective agent. Biochem. Biophys. Res. Commun. 368, 181–185. https://doi.org/10.1016/j.bbrc.2008.01.063

Taniguchi, C.M., Emanuelli, B., Kahn, C.R., 2006. Critical nodes in signalling pathways: insights into insulin action. Nat. Rev. Mol. Cell Biol. 7, 85–96. https://doi.org/10.1038/nrm1837

Tartaglia, L.A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G.J., Campfield, L.A., Clark, F.T., Deeds, J., Muir, C., Sanker, S., Moriarty, A., Moore, K.J., Smutko, J.S., Mays, G.G., Wool, E.A., Monroe, C.A., Tepper, R.I., 1995. Identification and expression cloning of a leptin receptor, OB-R. Cell 83, 1263–1271. https://doi.org/10.1016/0092-8674(95)90151-5

Tay, J., Thompson, C.H., Luscombe-Marsh, N.D., Wycherley, T.P., Noakes, M., Buckley, J.D., Wittert, G.A., Yancy, W.S.J., Brinkworth, G.D., 2018. Effects of an energy-restricted low-carbohydrate, high unsaturated fat/low saturated fat diet versus a high-carbohydrate, low-

fat diet in type 2 diabetes: A 2-year randomized clinical trial. Diabetes. Obes. Metab. 20, 858–871. https://doi.org/10.1111/dom.13164

Tchkonia, T., Giorgadze, N., Pirtskhalava, T., Tchoukalova, Y., Karagiannides, I., Forse, R.A., DePonte, M., Stevenson, M., Guo, W., Han, J., Waloga, G., Lash, T.L., Jensen, M.D., Kirkland, J.L., 2002. Fat depot origin affects adipogenesis in primary cultured and cloned human preadipocytes. Am. J. Physiol. Regul. Integr. Comp. Physiol. 282, R1286-96. https://doi.org/10.1152/ajpregu.00653.2001

Thon, M., Hosoi, T., Ozawa, K., 2016. Possible Integrative Actions of Leptin and Insulin Signaling in the Hypothalamus Targeting Energy Homeostasis. Front. Endocrinol. (Lausanne). 7, 138. https://doi.org/10.3389/fendo.2016.00138

Timper, K., Brüning, J.C., 2017. Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity. Dis. Model. Mech. 10, 679–689. https://doi.org/10.1242/dmm.026609

Trujillo, M.E., Scherer, P.E., 2006. Adipose tissue-derived factors: impact on health and disease. Endocr. Rev. 27, 762–778. https://doi.org/10.1210/er.2006-0033

Tvrzicka, E., Kremmyda, L.-S., Stankova, B., Zak, A., 2011. Fatty acids as biocompounds: their role in human metabolism, health and disease--a review. Part 1: classification, dietary sources and biological functions. Biomed. Pap. Med. Fac. Univ. Palacky, Olomouc, Czechoslov. 155, 117–130. https://doi.org/10.5507/bp.2011.038

Unger, R.H., 2002. Lipotoxic diseases. Annu. Rev. Med. 53, 319–336. https://doi.org/10.1146/annurev.med.53.082901.104057

Vaisse, C., Halaas, J.L., Horvath, C.M., Darnell, J.E.J., Stoffel, M., Friedman, J.M., 1996. Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. Nat. Genet. 14, 95–97. https://doi.org/10.1038/ng0996-95

Valladolid-Acebes, I., Fole, A., Martín, M., Morales, L., Victoria Cano, M., Ruiz-Gayo, M., Olmo, N. Del, 2013. Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin? Neurobiol. Learn. Mem. 106, 18–25. https://doi.org/10.1016/j.nlm.2013.06.012

Valladolid-Acebes, I., Merino, B., Principato, A., Fole, A., Barbas, C., Lorenzo, M.P., García, A., del Olmo, N., Ruiz-Gayo, M., Cano, V., 2012. High-fat diets induce changes in hippocampal glutamate metabolism and neurotransmission. Am. J. Physiol. - Endocrinol. Metab. 302, 396–402. https://doi.org/10.1152/ajpendo.00343.2011

Valladolid-Acebes, I, Stucchi, P., Cano, V., Fernández-Alfonso, M.S., Merino, B., Gil-Ortega, M., Fole, A., Morales, L., Ruiz-Gayo, M., Del Olmo, N., 2011. High-fat diets impair spatial learning in the radial-arm maze in mice. Neurobiol. Learn. Mem. 95, 80–85. https://doi.org/10.1016/j.nlm.2010.11.007

Valladolid-Acebes, I., Stucchi, P., Cano, V., Fernández-Alfonso, M.S., Merino, B., Gil-Ortega, M., Fole, A., Morales, L., Ruiz-Gayo, M., Olmo, N. Del, 2011. High-fat diets impair spatial learning in the radial-arm maze in mice. Neurobiol. Learn. Mem. 95, 80–85. https://doi.org/10.1016/j.nlm.2010.11.007

Van-Horn, M.R., Sild, M., Ruthazer, E.S., 2013. D-serine as a gliotransmitter and its roles in brain development and disease. Front. Cell. Neurosci. 7, 1–13. https://doi.org/10.3389/fncel.2013.00039

van den Berg, E., Kloppenborg, R.P., Kessels, R.P.C., Kappelle, L.J., Biessels, G.J., 2009. Type 2 diabetes mellitus, hypertension, dyslipidemia and obesity: A systematic comparison of their impact on cognition. Biochim. Biophys. Acta 1792, 470–481. https://doi.org/10.1016/j.bbadis.2008.09.004

Van Doorn, C., Macht, V.A., Grillo, C.A., Reagan, L.P., 2017. Leptin resistance and hippocampal behavioral deficits. Physiol. Behav. 176, 207–213. https://doi.org/10.1016/j.physbeh.2017.03.002

Vasselli, J.R., Scarpace, P.J., Harris, R.B.S., Banks, W.A., 2013. Dietary components in the development of leptin resistance. Adv. Nutr. 4, 164–175. https://doi.org/10.3945/an.112.003152

Vázquez-Vela, M.E.F., Torres, N., Tovar, A.R., 2008. White adipose tissue as endocrine organ and its role in obesity. Arch. Med. Res. 39, 715–728. https://doi.org/10.1016/j.arcmed.2008.09.005

Vázquez, I.A., Zapico, R.B., Rodríguez, C.F., 2007. La obesidad infantil como resultado de un estilo de vida obesogénico. Endocrinol. y Nutr. 54, 530–534. https://doi.org/10.1016/S1575-0922(07)71500-0

Virtue, S., Vidal-Puig, A., 2008. It's not how fat you are, it's what you do with it that counts. PLoS Biol. 6, e237. https://doi.org/10.1371/journal.pbio.0060237

Wagoner, B., Hausman, D.B., Harris, R.B.S., 2006. Direct and indirect effects of leptin on preadipocyte proliferation and differentiation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 290, R1557-64. https://doi.org/10.1152/ajpregu.00860.2005

Wajchenberg, B.L., 2000. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr. Rev. 21, 697–738. https://doi.org/10.1210/edrv.21.6.0415

Wang, M.-Y., Grayburn, P., Chen, S., Ravazzola, M., Orci, L., Unger, R.H., 2008. Adipogenic capacity and the susceptibility to type 2 diabetes and metabolic syndrome. Proc. Natl. Acad. Sci. U. S. A. 105, 6139–6144. https://doi.org/10.1073/pnas.0801981105

Wang, M.-Y., Orci, L., Ravazzola, M., Unger, R.H., 2005. Fat storage in adipocytes requires inactivation of leptin's paracrine activity: implications for treatment of human obesity. Proc.

Natl. Acad. Sci. U. S. A. 102, 18011–18016. https://doi.org/10.1073/pnas.0509001102

Wang, Y., Hsuchou, H., He, Y., Kastin, A.J., Pan, W., 2015. Role of Astrocytes in Leptin Signaling. J. Mol. Neurosci. 56, 829–839. https://doi.org/10.1007/s12031-015-0518-5

Wang, Z., Zhou, Y.T., Kakuma, T., Lee, Y., Kalra, S.P., Kalra, P.S., Pan, W., Unger, R.H., 2000. Leptin resistance of adipocytes in obesity: role of suppressors of cytokine signaling. Biochem. Biophys. Res. Commun. 277, 20–26. https://doi.org/10.1006/bbrc.2000.3615

Wayner, M.J., Armstrong, D.L., Phelix, C.F., Oomura, Y., 2004. Orexin-A (Hypocretin-1) and leptin enhance LTP in the dentate gyrus of rats in vivo. Peptides 25, 991–996. https://doi.org/10.1016/j.peptides.2004.03.018

Westra, M., Gutierrez, Y., MacGillavry, H.D., 2021. Contribution of Membrane Lipids to Postsynaptic Protein Organization. Front. Synaptic Neurosci. 13, 790773. https://doi.org/10.3389/fnsyn.2021.790773

WHO, 2021a. Obesity and overweight [WWW Document]. URL https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed 4.11.22).

WHO, 2021b. Noncommunicable diseases [WWW Document]. URL https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases (accessed 4.11.22).

Witter, M.P., 2007. Intrinsic and extrinsic wiring of CA3: indications for connectional heterogeneity. Learn. Mem. 14, 705–713. https://doi.org/10.1101/lm.725207

Woods, S.C., Schwartz, M.W., Baskin, D.G., Seeley, R.J., 2000. Food intake and the regulation of body weight. Annu. Rev. Psychol. 51, 255–277. https://doi.org/10.1146/annurev.psych.51.1.255

Wysoczański, T., Sokoła-Wysoczańska, E., Pękala, J., Lochyński, S., Czyż, K., Bodkowski, R., Herbinger, G., Patkowska-Sokoła, B., Librowski, T., 2016. Omega-3 Fatty Acids and their Role in Central Nervous System - A Review. Curr. Med. Chem. 23, 816–831. https://doi.org/10.2174/0929867323666160122114439

Xia, J., Yu, P., Zeng, Z., Ma, M., Zhang, G., Wan, D., Gong, D., Deng, S., Wang, J., 2021. Lauric Triglyceride Ameliorates High-Fat-Diet-Induced Obesity in Rats by Reducing Lipogenesis and Increasing Lipolysis and β-Oxidation. J. Agric. Food Chem. 69, 9157–9166. https://doi.org/10.1021/acs.jafc.0c07342

Yashiro, K., Philpot, B.D., 2008. Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. Neuropharmacology 55, 1081–1094. https://doi.org/10.1016/j.neuropharm.2008.07.046

Youssef, J., Badr, M., 2004. Role of Peroxisome Proliferator-Activated Receptors in Inflammation Control. J. Biomed. Biotechnol. 2004, 156–166. https://doi.org/10.1155/S1110724304308065

Yudkin, J.S., Eringa, E., Stehouwer, C.D.A., 2005. “Vasocrine” signalling from perivascular fat: a mechanism linking insulin resistance to vascular disease. Lancet (London, England) 365, 1817–1820. https://doi.org/10.1016/S0140-6736(05)66585-3

Zald, D.H., 2009. Orbitofrontal cortex contributions to food selection and decision making. Ann. Behav. Med. 38 Suppl 1, S18-24. https://doi.org/10.1007/s12160-009-9117-4

Zhang, F., Chen, Y., Heiman, M., Dimarchi, R., 2005. Leptin: structure, function and biology. Vitam. Horm. 71, 345–372. https://doi.org/10.1016/S0083-6729(05)71012-8

Zhang, W., Cline, M.A., Gilbert, E.R., 2014. Hypothalamus-adipose tissue crosstalk: neuropeptide Y and the regulation of energy metabolism. Nutr. Metab. (Lond). 11, 27. https://doi.org/10.1186/1743-7075-11-27

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., Friedman, J.M., 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425–432. https://doi.org/10.1038/372425a0

Zhao, F., Siu, J.J., Huang, W., Askwith, C., Cao, L., 2019. Insulin Modulates Excitatory Synaptic Transmission and Synaptic Plasticity in the Mouse Hippocampus. Neuroscience 411, 237–254. https://doi.org/10.1016/j.neuroscience.2019.05.033

ANEXOS

ANEXO 1

Título: *Saturated and unsaturated triglyceride-enriched diets modify amino acid content in the mice hippocampus*.

Autores: Jesús Fernández Felipe, Ana Belén Sanz Martos, Alberto Marcos, María Paz Lorenzo, Victoria Cano, Beatriz Merino, Emilio Ambrosio, Nuria del Olmo y Mariano Ruiz Gayo.

Publicado en: *Neuroscience letters* (ISSN: 0304-3940)

Factor de impacto: 3.197

Cuartil: Q3 (neurociencia)

Fecha de publicación: 2022

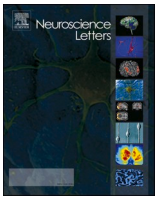
DOI: 10.1016/j.neulet.2022.136972

Neuroscience Letters 793 (2023) 136972

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet



Saturated and unsaturated triglyceride-enriched diets modify amino acid content in the mice hippocampus

Jesús Fernández-Felipe [a], Ana B. Sanz-Martos [b], Alberto Marcos [b], María P. Lorenzo [c], Victoria Cano [a], Beatriz Merino [a], Emilio Ambrosio [b], Nuria Del Olmo [b], Mariano Ruiz-Gayo [a,*]

[a] Department of Health and Pharmaceutical Sciences, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain
[b] Department of Psychobiology, Facultad de Psicología, Universidad Nacional de Educación a Distancia, Madrid, Spain
[c] Center for Metabolomics and Bioanalysis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain

ARTICLE INFO

Keywords:
Saturated fat
Unsaturated fat
Neurotransmitters
L-Glutamate
D-Serine

ABSTRACT

Elevated intake of fat modulates L-glutamate (L-Glu) turnover within the hippocampus (HIP). Our aim has been to investigate the effect of saturated *vs* unsaturated fat on the content of L-Glu and other amino acids involved in synaptic transmission within the HIP. The study was carried out in male mice fed (2 h or 8 weeks) with standard chow or with diets enriched either with saturated (SOLF) or unsaturated triglycerides (UOLF). An *in vitro* assay was performed in HIP slices incubated with palmitic (PA), oleic (OA), or lauric acid (LA). Amino acids were quantified by capillary electrophoresis. While both diets increased the amount of L-Glu and L-aspartate and decreased L-glutamine levels, only UOLF affected D-serine and taurine levels. γ-Aminobutyric acid was specifically decreased by SOLF. *In vitro* assays revealed that PA and OA modified L-Glu, glycine, L-serine and D-serine concentration. Our results suggest that fatty acids contained in SOLF and UOLF have an impact on HIP amino acid turnover that may account, at least partially, for the functional changes evoked by these diets.

1. Introduction

Regular intake of diets enriched in both saturated and unsaturated triglycerides impairs synaptic plasticity within the hippocampus (HIP) [1,2] by a mechanism associated with changes in L-glutamate (L-Glu) synthesis and turnover [2]. L-Glu metabolism is interconnected with that of γ-aminobutyric acid (GABA) and L-glutamine (L-Gln) and involves neurons and astrocytes [3]. L-serine (L-Ser), D-serine (D-Ser), and glycine (Gly) are also pivotal modulators of glutamatergic transmission eventually affected by nutritional inputs [4]. On the other hand, taurine (Tau) is endowed with an inhibitory effect on synaptic transmission by modulating GABA and Gly receptors [5] and it has been shown to be increased by high-fat diets (HFD) in different brain areas, including the HIP [6]. Despite all this research the specific contribution of saturated *vs* unsaturated fat to HIP amino acids has not been investigated.

Our hypothesis is that these types of fat differently affect HIP amino acid concentration. This assumption is based on previous studies showing that these diets have a different impact on HIP synaptic transmission and plasticity [1], which is compatible with changes in the level of amino acids involved in neurotransmission.

To assess this hypothesis, HIP amino acids were analyzed in mice that consumed HFD containing 40 % of either saturated (palm kernel oil, containing 60 % of lauric and palmitic acids) or unsaturated fat (high-oleic sunflower oil; greater than75 % oleic acid) during either 2-h or 8 weeks. To our knowledge this is the first attempt to investigate the effect of these fats on HIP amino acids. An *in vitro* study was performed in HIP slices incubated with saturated/unsaturated fatty acids (FA) to investigate the contribution of specific FA.

2. Material and methods

2.1. Animals and diets

Assays were carried out in C57BL male mice (Charles River, France)

Abbreviations: BCA, Bicinchoninic acid; CE, Capillary electrophoresis; FA, Fatty acid; GABA, γ-aminobutyric acid; HFD, High-fat diet; HIP, Hippocampus; KRB, Krebs-Ringer bicarbonate; LA, Lauric acid; NBD-F, 4-fluoro-7-nitrobenzofurazan; OA, Oleic acid; PA, Palmitic acid; SOLF, Saturated oil-enriched food; UOLF, Unsaturated oil-enriched food.

* Corresponding author at: Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad San Pablo-CEU, Campus de Montepríncipe, 28668 Madrid, Spain.
E-mail address: ruigayo@ceu.es (M. Ruiz-Gayo).

https://doi.org/10.1016/j.neulet.2022.136972
Received 31 May 2022; Received in revised form 10 November 2022; Accepted 16 November 2022
Available online 19 November 2022

that consumed the experimental diets from postnatal day 35. Four-week-old animals were singly housed under a 12-h light (08:00–20:00)/12-h dark cycle, in a temperature-controlled room (22 °C) with free access to water and rodent chow, in accordance with the European Communities Council Directive (86/609/EEC) for the care and use of laboratory animals (PCD-CEU08-112–16). After 1-week acclimation, animals with similar average body weight (BW) were randomly assigned to one of three experimental groups fed either with standard chow (SD, Teklad global 2018, Harlan Laboratories, IN), standard chow enriched with 40 % of either high-oleic sunflower oil (***Unsaturated OiL-enriched Food***, UOLF) or 40 % palm seed oil (***Saturated OiL-enriched Food***, SOLF). Animals consumed these diets during either 2 h or 8 weeks. Both UOLF and SOLF were manufactured in our animal facility as already described [1,7].

In the **2-h assay**, diets were provided between 06:00 and 08:00, after 12 h fasting. In the **8-week assay**, animals consumed the diets *ad libitum* for 8 weeks. At the end of the treatment mice were killed by decapitation under isoflurane anaesthesia and HIP were dissected. Biochemical data of animals consuming SOLF/UOLF (8 weeks) have been previously reported [1,7].

2.2. In vitro assay

In vitro assays were carried out in HIP slices obtained from naïve five-week-old C57BL male mice (Charles River, France) (n = 6–8 *per* group). Animals were killed by decapitation under isoflurane anaesthesia, and transverse HIP slices (400 μm; (Stoelting Tissue Slicer; Stoelting Europe, Dublin, Ireland) transferred to 24-well plates containing KRB (20–22 °C; 119 mM NaCl, 2.5 mM KCl, 1 mM KH_2PO_4, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 26.2 mM $NaHCO_3$, 11 mM glucose) [1]. After 2-h stabilization, KRB was supplemented (30, 75 and 300 μM) with either palmitic (PA), lauric (LA) or oleic acids (OA) as well as with mixtures of LA/PA (3:1) and incubated for 2-h. The medium was bubbled with O_2/CO_2 (95/5).

2.3. Sample handling and derivatization with NBD-F

Whole HIP or HIP slices were homogenized in a TissueLyser (50 Hz × 1 min; Qiagen Spain) in 500 μL or 150 μL of ice-cold KRB, respectively. After centrifugation (3000 g, 30 min), supernatants were collected and protein concentration quantified (BCA protein assay kit; Fisher Scientific, Spain). For derivatization, samples (45 μL) were mixed (60 °C; 17 min in darkness) with 15 μL of 150 μM α-aminoadipic acid as external standard (Merck, Germany), 40 μL of 40 mM 4-fluoro-7-nitrobenzofurazan (NBD-F; Cymit Química, Spain) in methanol:0.5 mM HCl (1:1), and 150 μL of 10 mM borate buffer (pH = 9.0).

2.4. Determination of amino acids by capillary electrophoresis

A capillary electrophoresis (CE) method, developed by Lorenzo *et al.*, 2013 [8], was employed to determine amino acid concentrations. Analyses were carried out in a P/ACE MDQ system (Beckman-Coulter PA 800plus, Fullerton, CA, USA), equipped with a solid-state (diode) laser-induced fluorescence detector, operating at λ_{exc}:488 nm and λ_{em}:520 nm. The capillary used consisted in an uncoated fused silica capillary (Trajan Scientific, Australia) (60 cm in total length; 50 cm effective length), with an internal diameter of 75 μM. Capillary was rinsed between runs (5 min, 20 psi) with 50 % methanol/H_2O (3 min), 0.5 M NaOH (3 min), H_2O (2 min) and running buffer (12.5 mM native β-cyclodextrin and 90 mM borate buffer, pH = 10.25, supplemented with 2 % methanol). Sample injection was made at the anode by pressure at 0.5 psi for 17 s. The voltage applied was 21 kV and current observed under these conditions was approximately 70 μA. Every-five analysis, running buffer and the other conditioning solutions were refreshed for maintaining CE features. Detection was made at the cathode. The capillary temperature was kept at 17 °C and samples were kept under refrigeration (7 ± 2 °C). Calibration curves were constructed to allow proper sample quantification and values were expressed as mg of amino acid *per* g of total protein (mg/g). Peak areas were normalized by migration time. The resulting corrected area was divided by the corrected peak area corresponding to the external standard.

2.5. Statistics

Data were analysed by 1-way ANOVA with *post-hoc* Bonferroni correction. Data were expressed as means ± S.E.M. and statistical significance was set at $P < 0.05$. Normal distribution and variance homogeneity were assessed by means of the Bartlett and Brown-Forsyte test. All statistics were performed using GraphPad Prism software (GraphPad Software Inc. USA; Version 9.0a).

3. Results

3.1. Effect of dietary treatment on food intake

As illustrated in Fig. 1A, 2-h dietary intervention did not affect energy intake. In contrast, two-way repeated measures ANOVA revealed an effect of dietary treatment ($F_{(2,248)} = 4{,}08$; $P < 0.01$) and time ($F_{(7,248)} = 11{,}02$; $P < 0.01$) as well as a significant interaction dietary treatment × time ($F_{(14,248)} = 2{,}48$; $P < 0.01$) in the eight-week treatment cohort (Fig. 1B). Accumulated nutrient intake appears summarized in Fig. 1C. One-way ANOVA indicated a diet effect in carbohydrate ($F_{(2,36)} = 170$; $P < 0.001$), protein ($F_{(2,36)} = 176.1$; $P < 0.001$) and fat intake ($F_{(2,36)} = 638.9$; $P < 0.001$).

3.2. Effect of 8-week dietary treatment with SOLF and UOLF on amino acid content in the HIP

As illustrated in Fig. 2, both SOLF and UOLF increased L-Glu ($P < 0.01$; Fig. 2A) and L-Asp ($P < 0.01$; Fig. 2C) and reduced L-Gln content ($P < 0.05$; Fig. 2B), whereas GABA was only reduced by SOLF ($P < 0.001$; Fig. 2G). Moreover, D-Ser levels were reduced by UOLF ($P < 0.05$; Fig. 2E). L-Ser levels were not affected by the dietary treatment, while the D-Ser/L-Ser ratio was specifically reduced by UOLF ($P < 0.05$; Fig. 2F). Tau concentration weas increased by UOLF ($P < 0.001$; Fig. 2I). Gly levels were not modified by the diets (Fig. 2H).

3.3. Effect of 2-h dietary treatment with SOLF and UOLF on amino acid content in the HIP

Two-hour dietary treatment did not modify L-Glu levels but decreased L-Asp ($P < 0.05$) and L-Gln levels ($P < 0.001$) (Fig. 3A-C). As already observed after 8-week diet consumption, D-Ser and D/L-Ser ratios were reduced ($P < 0.01$) specifically in UOLF mice (Fig. 3D-F). No changes were observed either in GABA, Gly or Tau levels (Fig. 3G-I).

3.4. In vitro effect of lauric, palmitic and oleic acids on amino acid content in hippocampus slices

Since the diets used in the *in vivo* study contain elevated amounts of PA and LA (these FA account for more than 60 % of the total FA content of SOLF) or OA (75 % of UOLF FA content)[7], the *in vitro* effect of PA, LA and OA was analysed in HIP slices. A 3:1 combination of LA:PA, corresponding to the ratio of these FA present in SOLF, was also used. The results appear summarized in Table 1.

PA, OA, as well as the combination LA:PA, reduced both D-Ser and L-Ser, regardless the FA concentration used in the assay ($P < 0.001$). No changes were observed in D/L-Ser ratios. LA only decreased D-Ser levels ($P < 0.05$). The concentration of L-Glu was altered by PA ($P < 0.01$), OA ($P < 0.01$) and LA:PA ($P < 0.001$), while LA did not modify L-Glu levels. Concerning GABA levels, all PA, LA and OA were without effect, while the Gly concentration was reduced by PA and OA (100 μM) as well as by the combination LA:PA ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively).

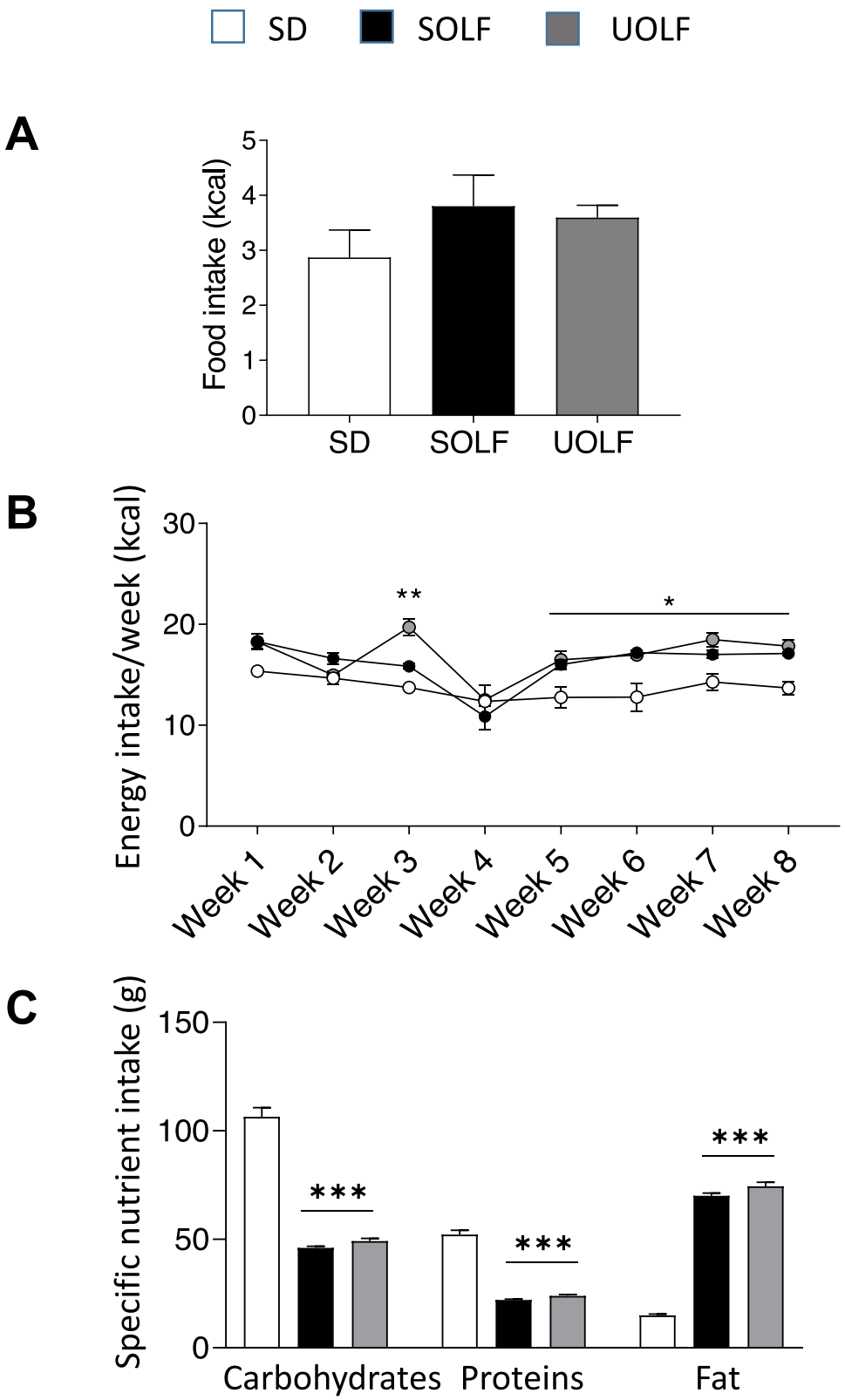


Fig. 1. Energy intake in mice consuming either SD, SOLF or UOLF during 2 h (A) or eight weeks (B). Accumulated nutrient intake was also measured during 8 weeks (C). Data are means ± S.E.M. (n = 6–8, in the 2-h treatment assay; n = 10–12 in the 8-week treatment assay). For statistical analysis, 1-way (A and C) or two-way ANOVA (B) were performed. ***P < 0.001, **P < 0.01 and *P < 0.05 *vs* SD group (Bonferroni's post hoc test).

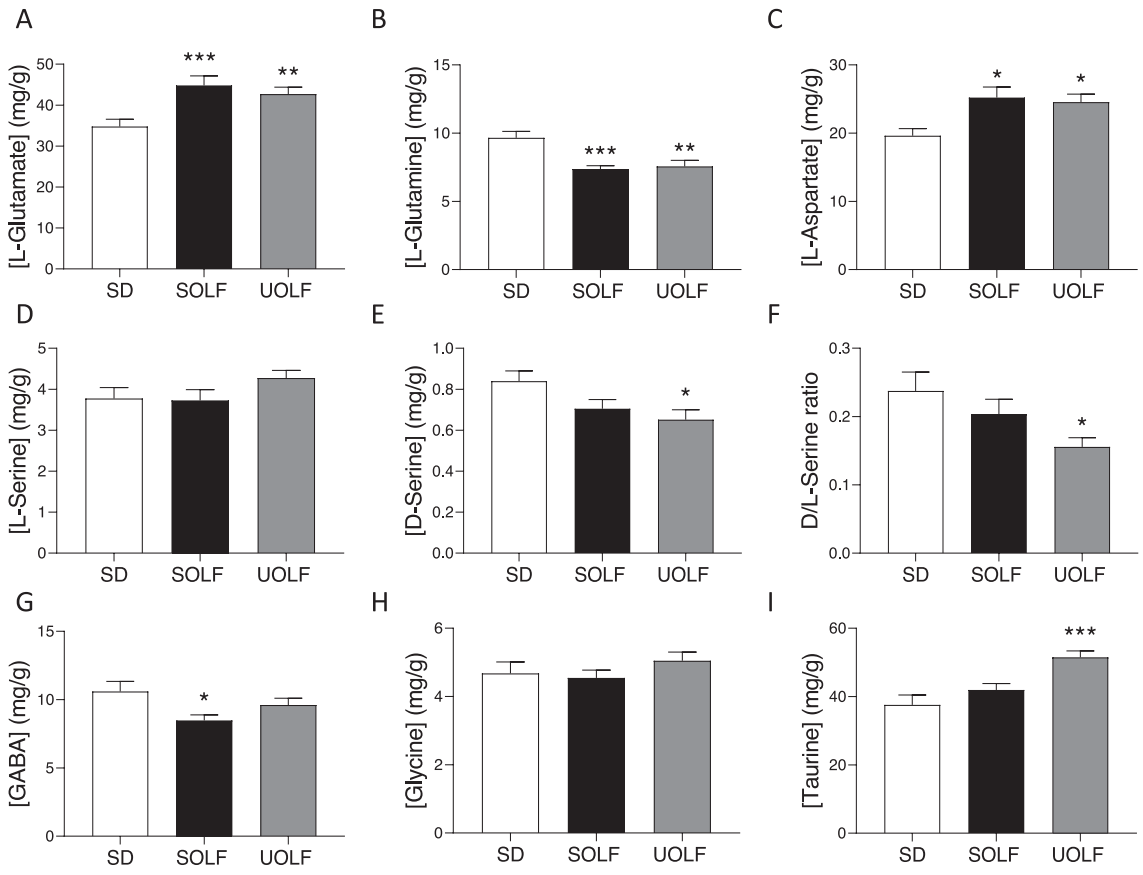


Fig. 2. Effect of eight-week feeding with SOLF and UOLF on amino acid content in the HIP. Panels A-I illustrate the influence of SOLF and UOLF on L-Glu (A), L-Gln (B), L-Asp (C), L-Ser (D), D-Ser (E), D/L-Ser ratio (F), GABA (G), Gly (H) and Tau (I). Data are means ± S.E.M. (mg/g protein). For statistical analysis, 1-way ANOVA was performed (n = 10–12/ group) followed by Bonferroni post hoc test when necessary. ***P < 0.001, **P < 0.01, *P < 0.05, compared to SD.

No effect was found in the case of Tau.

L-Gln was not analysed in the *in vitro* study because it co-eluted with L and D-leucine under our experimental conditions.

4. Discussion

The current study shows that chronic intake (8 weeks) of diets enriched with saturated (SOLF) or unsaturated fat (UOLF) modulate the content of amino acids relevant for HIP neurotransmission.

Thus, chronic intake of SOLF and UOLF increased both L-Glu and L-Asp, and decreased L-Gln concentrations. This effect might be secondary to the rise of acetyl-CoA derived from FA β-oxidation, which could theoretically fuel the tricarboxylic acid (TCA) cycle, and therefore promote L-Glu synthesis [9]. This circumstance has been previously observed in other studies carried out with other HFDs [6,10,11]. From our data we cannot identify the origin of L-Glu, but taken into account the limited β-oxidation rate in neurons [12], we hypothesize that the main source of L-Glu is astrocytes [13]. The decrease of L-Gln levels is unexpected since this amino acid derives from L-Glu. As a possibility, we hypothesize that SOLF/UOLF might decrease the expression and/or activity of glutamine synthase, as already observed in previous studies carried out with classical HFDs [2]. Otherwise, the possibility of an enhanced expression/activity of glutaminase cannot be ruled out, although, to our knowledge, there are no data in the literature regarding the influence of HFD on this enzyme. Interestingly, an up-regulation of glutaminase has been shown to cause HIP-dependent learning deficits [14], which have been also detected in animals consuming SOLF [1]. In contrast to the results obtained after 8-week dietary treatment, 2-h consumption of SOLF/UOLF, failed to modify L-Glu levels (although it reduced both L-Gln and L-Asp concentrations) indicating that acute and chronic effects of these diets differ notably. Concerning the *in vitro* effect of FA, the rise of L-Glu triggered by PA and OA is coherent with an acute overload of the TCA cycle [15]. The effect of the combination LA:PA was similar to that of PA alone, suggesting that changes evoked by SOLF could be related to the elevated PA content on this diet. This finding is coherent with the increase of L-Glu induced by PA, observed by other authors in *in vivo* studies [16].

The downregulation of GABA elicited by SOLF is striking since GABA derives from L-Glu and, therefore, an increase rather than a decrease of GABA would be expected [17]. Our finding suggests that SOLF might decrease glutamate decarboxylase (GAD; the enzyme that accounts for GABA synthesis from L-Glu) expression and/or activity. This is coherent with previous findings of our group showing that HFD downregulate GAD expression within the HIP [2]. Nevertheless, this possibility is uncertain since HFD similar to that used in our study have been shown to enhance GAD activity [18,19]. Further research aimed at identifying possible changes in GAD expression/activity by SOLF/UOLF should be carried out.

Another apparent target of saturated and unsaturated FA are L- and D-Ser. L-Ser is a key element in astrocyte metabolism since it is the precursor of both Gly [20–22] and D-Ser [23], and is also necessary for ceramide synthesis [24–26]. Under our conditions, L-Ser content was not modified by *in vivo* SOLF/UOLF, although it was reduced *in vitro* by PA and OA as well as by LA:PA. As a possible explanation, we speculate that an excess of PA and OA might activate ceramide synthesis [27–29] and therefore consume L-Ser; the lack of effect observed in the *in vivo* study suggests that the modulation of L-Ser turnover might be more effective *in vitro* than *in vivo,* probably because L-Ser can be provided by nutritional

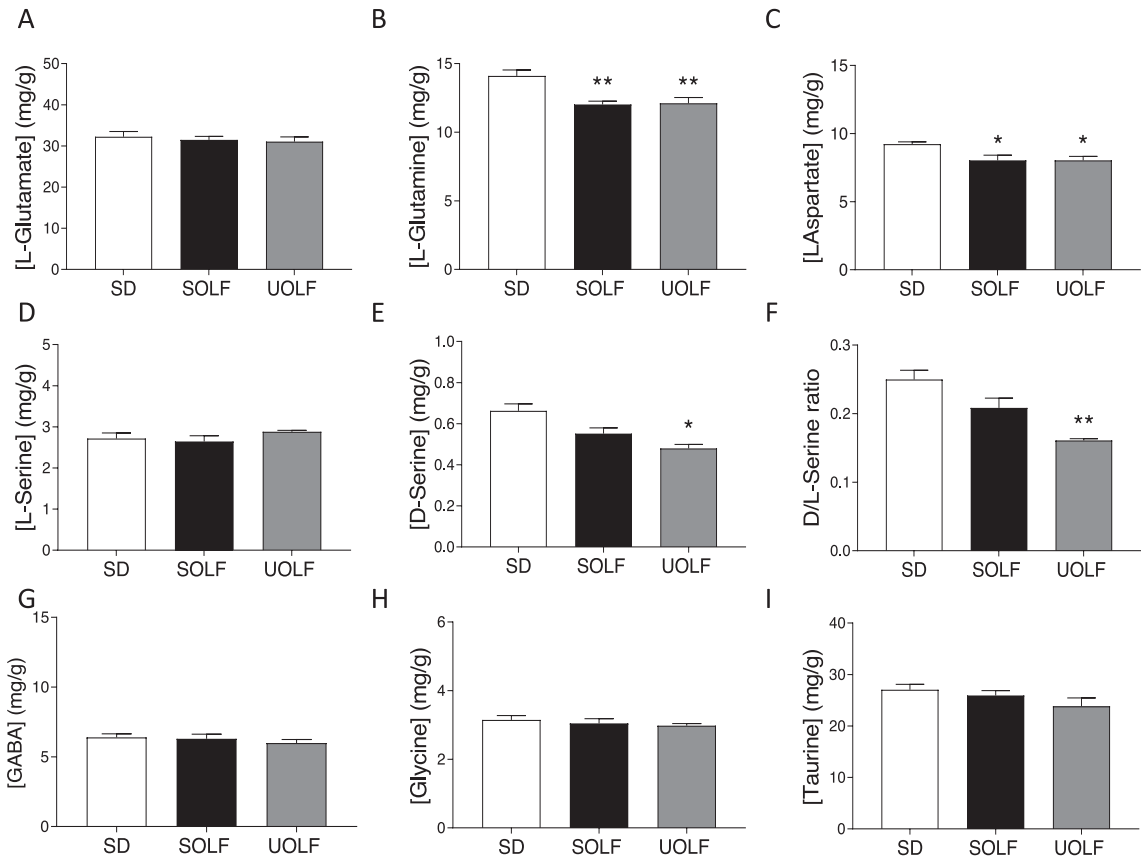


Fig. 3. Effect of 2-hour consumption of SOLF and UOLF on amino acid content in the HIP. Panels A-I illustrate the influence of SOLF and UOLF on L-Glu (A), L-Gln (B), L-Asp (C), L-Ser (D), D-Ser (E), D/L-Ser ratio (F), GABA (G), Gly (H) and Tau (I). Data are means ± S.E.M. (mg/g protein). For statistical analysis, 1-way ANOVA was performed (n = 8/group) followed by Bonferroni post hoc test when necessary. **P < 0.01 and *P < 0.05, compared to SD.

Table 1
Amino acid content in HIP slices treated with palmitic acid (PA), oleic acid (OA), lauric acid (LA) or LA:PA (3:1). Data are means ± S.E.M. (mg/g protein). For statistical analysis, 1-way ANOVA was performed (n = 6–8 slices per group) followed by Bonferroni *post hoc* test when necessary. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, compared to controls.

		Amino acids (mg/g)							
		L-Ser	D-Ser	D-Ser/L-Ser	GABA	Gly	Tau	L-Glu	L-Asp
Control		1.94 ± 0.12	0.25 ± 0.01	0.12 ± 0.01	1.92 ± 0.21	1.12 ± 0.11	4.88 ± 0.41	9.47 ± 0.66	3.49 ± 0.27
[PA]	**30**	**1.19 ± 0.21***	**0.09 ± 0.02****	0.13 ± 0.01	2.13 ± 0.22	0.89 ± 0.09	6.41 ± 0.62	**17.09 ± 0.87****	5.21 ± 0.22
μM	**75**	**0.94 ± 0.09*****	**0.09 ± 0.0*****	0.14 ± 0.01	1.99 ± 0.43	0.81 ± 0.11	5.43 ± 0.41	**13.77 ± 1.12***	4.14 ± 0.44
	300	**0.61 ± 0.08*****	**0.05 ± 0.01*****	0.13 ± 0.01	1.91 ± 0.33	**0.69 ± 0.09***	3.58 ± 0.48	9.26 ± 1.07	3.13 ± 0.37
[OA]	**30**	**1.14 ± 0.22****	**0.14 ± 0.09****	0.11 ± 0.01	1.87 ± 0.27	0.82 ± 0.09	5.43 ± 0.61	**14.37 ± 1.16***	5.38 ± 0.47
μM	**75**	**0.93 ± 0.09*****	**0.11 ± 0.09*****	0.09 ± 0.02	1.68 ± 0.25	0.73 ± 0.09	5.06 ± 0.48	13.16 ± 1.07	4.38 ± 0.39
	300	**0.62 ± 0.07*****	**0.06 ± 0.01*****	0.12 ± 0.02	1.71 ± 0.19	**0.66 ± 0.11***	3.88 ± 0.27	9.37 ± 1.42	2.37 ± 0.16
[LA]	**30**	1.55 ± 0.11	**0.16 ± 0.02***	0.15 ± 0.01	1.69 ± 0.17	1.12 ± 0.21	3.19 ± 0.22	10.01 ± 1.36	4.07 ± 0.51
μM	**75**	1.39 ± 0.12	**0.17 ± 0.01***	0.14 ± 0.02	1.87 ± 0.18	0.84 ± 0.11	3.87 ± 0.31	10.66 ± 1.19	3.69 ± 0.17
	300	1.38 ± 0.11	**0.15 ± 0.02****	0.14 ± 0.01	1.64 ± 0.25	0.74 ± 0.09	6.26 ± 0.09	11.79 ± 1.47	4.46 ± 0.36
[LA] / [PA]	**30 (22.5/7.5)**	**1.19 ± 0.11***	**0.09 ± 0.02****	0.11 ± 0.02	1.63 ± 0.16	0.98 ± 0.09	4.41 ± 0.87	**17.02 ± 1.73****	5.29 ± 0.71
(3:1)	**75 (56.3/18.7)**	**0.95 ± 0.09*****	**0.05 ± 0.01*****	0.07 ± 0.01	1.54 ± 0.09	0.74 ± 0.08	3.69 ± 0.17	12.26 ± 1.12	3.72 ± 0.81
μM	**300 (225/75)**	**0.72 ± 0.09*****	**0.03 ± 0.01*****	0.08 ± 0.01	1.57 ± 0.11	**0.51 ± 0.09****	6.77 ± 0.36	9.94 ± 1.43	2.23 ± 0.29

sources. In any case, further studies will be necessary to clarify this issue.

In contrast to L-Ser, D-Ser levels were reduced both by 8-week and 2-h dietary treatment, although statistical significance was only detected in UOLF mice. Since D-Ser is mainly synthetized in astrocytes from L-Ser [30], a leakage of this amino acid toward ceramide synthesis might limit D-Ser synthesis. This is a very relevant finding since D-Ser is a co-agonist of the NMDA receptor [30], and we have recently reported that both UOLF and SOLF impair HIP synaptic plasticity [1]. Interestingly, a decrease of D-Ser was also observed in *in vitro* by PA, OA and LA, supporting the concept that the effect of UOLF on D-Ser levels is not linked to the specific FA composition of this diet. In fact, the poor effect of SOLF on D-Ser levels is of difficult interpretation since both LA and PA, which are main components of this diet, led to a reduction of D-Ser *in vitro*.

In regard to the effect of SOLF/UOLF on Tau levels, our data show that this molecule is not sensitive to saturated FA. The increase of Tau triggered by UOLF is coherent with the apparent neuroprotective effect

of OA [31,32]. In any case, the lack of effect detected in the *in vitro* study suggest that FA themselves are not directly involved in the *in vivo* effect of the diets.

We would like to highlight that both SOLF and UOLF contain significantly less proteins and carbohydrates than chow and, therefore, it cannot be excluded that an eventual deficiency of protein and/or carbohydrates may have an influence on HIP amino acid content. Moreover, mice on SOLF/UOLF displayed a small but significant hyperphagia during the last four weeks of the dietary treatment, which could contribute to the effects observed. The possibility of including pair-fed control groups to assess this eventuality was discarded since changes of feeding pattern evoked by this kind of interventions may provoke additional metabolic alterations [33]. In any case, our *in vitro* study strongly supports the hypothesis that FA themselves have an impact on amino acid content within the HIP.

In conclusion, we demonstrate that both SOLF and UOLF modulate the turnover of amino acids involved in neurotransmission, an effect that seems to depend, at least in part, on the FA contained in the diets.

Funding: This work was supported by Ministerio de Ciencia e Innovación (PID2020-117422RB-C21 and PID2020-117422RB-C22) and Fundación Universitaria San Pablo-CEU (FUSP), Spain. JF-F is supported by a grant from FUSP.

CRediT authorship contribution statement

Jesús Fernández-Felipe: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Ana B. Sanz-Martos:** Investigation. **Alberto Marcos:** Methodology, Formal analysis, Writing – original draft, Visualization. **María P. Lorenzo:** Methodology. **Victoria Cano:** Investigation. **Beatriz Merino:** Investigation. **Emilio Ambrosio:** Resources. **Nuria Del Olmo:** Conceptualization, Resources. **Mariano Ruiz-Gayo:** Conceptualization, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J. Fernández-Felipe, B. Merino, A.B. Sanz-Martos, A. Plaza, A. Contreras, V. Naranjo, L. Morales, J.A. Chowen, V. Cano, M. Ruiz-Gayo, N. Del Olmo, Saturated and unsaturated fat diets impair hippocampal glutamatergic transmission in adolescent mice, Psychoneuroendocrinology 133 (2021), 105429, https://doi.org/10.1016/j.psyneuen.2021.105429.

[2] I. Valladolid-Acebes, B. Merino, A. Principato, A. Fole, C. Barbas, M.P. Lorenzo, A. García, N. del Olmo, M. Ruiz-Gayo, V. Cano, High-fat diets induce changes in hippocampal glutamate metabolism and neurotransmission, Am. J. Physiol. - Endocrinol. Metab. 302 (2012) 396–402, https://doi.org/10.1152/ajpendo.00343.2011.

[3] L.K. Bak, A. Schousboe, H.S. Waagepetersen, The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer, J. Neurochem. 98 (2006) 641–653, https://doi.org/10.1111/j.1471-4159.2006.03913.x.

[4] N. Camargo, J.F. Brouwers, M. Loos, D.H. Gutmann, A.B. Smit, M.H.G. Verheijen, High-fat diet ameliorates neurological deficits caused by defective astrocyte lipid metabolism, FASEB J. Off. Publ. Fed. Am. Soc Exp. Biol. 26 (2012) 4302–4315, https://doi.org/10.1096/fj.12-205807.

[5] P.F. Urban, P. Reichert, P. Mandel, Taurine metabolism and function, Adv. Biochem. Psychopharmacol. 29 (1981) 537–544.

[6] B. Lizarbe, A.F. Soares, S. Larsson, J.M.N. Duarte, Neurochemical modifications in the hippocampus, cortex and hypothalamus of mice exposed to long-term high-fat diet, Front. Neurosci. 13 (2019) 1–15, https://doi.org/10.3389/fnins.2018.00985.

[7] A. Plaza, M. Antonazzi, J. Blanco-Urgoiti, N. Del Olmo, M. Ruiz-Gayo, Potential Role of Leptin in Cardiac Steatosis Induced by Highly Saturated Fat Intake during Adolescence, Mol. Nutr. Food Res. 63 (2019) 1–11, https://doi.org/10.1002/mnfr.201900110.

[8] M.P. Lorenzo, A. Navarrete, C. Balderas, A. Garcia, Optimization and validation of a CE-LIF method for amino acid determination in biological samples, J. Pharm. Biomed. Anal. 73 (2013) 116–124, https://doi.org/10.1016/j.jpba.2012.03.017.

[9] F.-M. Girault, S. Sonnay, R. Gruetter, J.M.N. Duarte, Alterations of Brain Energy Metabolism in Type 2 Diabetic Goto-Kakizaki Rats Measured In Vivo by (13)C Magnetic Resonance Spectroscopy, Neurotox. Res. 36 (2019) 268–278, https://doi.org/10.1007/s12640-017-9821-y.

[10] M. Ribeiro, J. Castelhano, L.I. Petrella, J. Sereno, T. Rodrigues, C. Neves, L. Letra, F.I. Baptista, R. Seiça, P. Matafome, M. Castelo-Branco, High-fat diet induces a neurometabolic state characterized by changes in glutamate and N-acetylaspartate pools associated with early glucose intolerance: An in vivo multimodal MRI study, J. Magn. Reson. Imaging. 48 (2018) 757–766, https://doi.org/10.1002/jmri.25942.

[11] R.S.M. Labban, H. Alfawaz, A.T. Almnaizel, W.M. Hassan, R.S. Bhat, N. M. Moubayed, G. Bjørklund, A. El-Ansary, High-fat diet-induced obesity and impairment of brain neurotransmitter pool, Transl. Neurosci. 11 (2020) 147–160, https://doi.org/10.1515/tnsci-2020-0099.

[12] J.P. Bolaños, Bioenergetics and redox adaptations of astrocytes to neuronal activity, J. Neurochem. 139 (2016) 115–125, https://doi.org/10.1111/jnc.13486.

[13] A. Falkowska, I. Gutowska, M. Goschorska, P. Nowacki, D. Chlubek, I. Baranowska-Bosiacka, Energy metabolism of the brain, including the cooperation between astrocytes and neurons, especially in the context of glycogen metabolism, Int. J. Mol. Sci. 16 (2015) 25959–25981, https://doi.org/10.3390/ijms161125939.

[14] Y. Wang, Y. Li, R. Zhao, B. Wu, B. Lanoha, Z. Tong, J. Peer, J. Liu, H. Xiong, Y. Huang, J. Zheng, Glutaminase C overexpression in the brain induces learning deficits, synaptic dysfunctions, and neuroinflammation in mice, Brain. Behav. Immun. 66 (2017) 135–145, https://doi.org/10.1016/j.bbi.2017.06.007.

[15] P. Honegger, O. Braissant, H. Henry, O. Boulat, C. Bachmann, M.G. Zurich, B. Pardo, Alteration of amino acid metabolism in neuronal aggregate cultures exposed to hypoglycaemic conditions, J. Neurochem. 81 (2002) 1141–1151, https://doi.org/10.1046/j.1471-4159.2002.00888.x.

[16] D. Osorio, A. Pinzón, C. Martín-Jiménez, G.E. Barreto, J. González, Multiple Pathways Involved in Palmitic Acid-Induced Toxicity: A System Biology Approach, Front. Neurosci. 13 (2020) 1–14, https://doi.org/10.3389/fnins.2019.01410.

[17] Y. Daikhin, M. Yudkoff, Ketone bodies and brain glutamate and GABA metabolism, Dev. Neurosci. 20 (1998) 358–364, https://doi.org/10.1159/000017331.

[18] M. Erecińska, D. Nelson, Y. Daikhin, M. Yudkoff, Regulation of GABA level in rat brain synaptosomes: fluxes through enzymes of the GABA shunt and effects of glutamate, calcium, and ketone bodies, J. Neurochem. 67 (1996) 2325–2334, https://doi.org/10.1046/j.1471-4159.1996.67062325.x.

[19] C.M. Cheng, K. Hicks, J. Wang, D.A. Eagles, C.A. Bondy, Caloric restriction augments brain glutamic acid decarboxylase-65 and -67 expression, J. Neurosci. Res. 77 (2004) 270–276, https://doi.org/10.1002/jnr.20144.

[20] S. Neame, H. Safory, I. Radzishevsky, A. Touitou, F. Marchesani, M. Marchetti, S. Kellner, S. Berlin, V.N. Foltyn, S. Engelender, J.M. Billard, H. Wolosker, The NMDA receptor activation by D-serine and glycine is controlled by an astrocytic Phgdh-dependent serine shuttle, Proc. Natl. Acad. Sci. U.S.A. 116 (2019) 20736–20742, https://doi.org/10.1073/pnas.1909458116.

[21] S. Furuya, T. Tabata, J. Mitoma, K. Yamada, M. Yamasaki, A. Makino, T. Yamamoto, M. Watanabe, M. Kano, Y. Hirabayashi, L-Serine and glycine serve as major astroglia-derived trophic factors for cerebellar Purkinje neurons, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 11528–11533, https://doi.org/10.1073/pnas.200364497.

[22] M. Maugard, P.A. Vigneron, J.P. Bolaños, G. Bonvento, L-Serine links metabolism with neurotransmission, Prog. Neurobiol. 197 (2021), https://doi.org/10.1016/j.pneurobio.2020.101896.

[23] M.R. Van-Horn, M. Sild, E.S. Ruthazer, D-serine as a gliotransmitter and its roles in brain development and disease, Front. Cell. Neurosci. 7 (2013) 1–13, https://doi.org/10.3389/fncel.2013.00039.

[24] G. Murtas, G.L. Marcone, S. Sacchi, L. Pollegioni, L-serine synthesis via the phosphorylated pathway in humans, Cell. Mol. Life Sci. 77 (2020) 5131–5148, https://doi.org/10.1007/s00018-020-03574-z.

[25] Y. Hirabayashi, S. Furuya, Roles of l-serine and sphingolipid synthesis in brain development and neuronal survival, Prog. Lipid Res. 47 (2008) 188–203, https://doi.org/10.1016/j.plipres.2008.01.003.

[26] O. Ben-David, A.H. Futerman, The role of the ceramide acyl chain length in neurodegeneration: involvement of ceramide synthases, Neuromolecular Med. 12 (2010) 341–350, https://doi.org/10.1007/s12017-010-8114-x.

[27] C. Blázquez, I. Galve-Roperh, M. Guzmán, De novo-synthesized ceramide signals apoptosis in astrocytes via extracellular signal-regulated kinase, FASEB J. Off. Publ. Fed. Am. Soc Exp. Biol. 14 (2000) 2315–2322, https://doi.org/10.1096/fj.00-0122com.

[28] S. Patil, J. Melrose, C. Chan, Involvement of astroglial ceramide in palmitic acid-induced Alzheimer-like changes in primary neurons, Eur. J. Neurosci. 26 (2007) 2131–2141, https://doi.org/10.1111/j.1460-9568.2007.05797.x.

[29] N. Itami, K. Shirasuna, T. Kuwayama, H. Iwata, Palmitic acid induces ceramide accumulation, mitochondrial protein hyperacetylation, and mitochondrial dysfunction in porcine oocytes, Biol. Reprod. 98 (2018) 644–653, https://doi.org/10.1093/biolre/ioy023.

[30] C. Henneberger, T. Papouin, S.H.R. Oliet, D.A. Rusakov, Long-term potentiation depends on release of d-serine from astrocytes, Nature 463 (2010) 232–236, https://doi.org/10.1038/nature08673.

[31] J. Beaulieu, G. Costa, J. Renaud, A. Moitié, H. Glémet, D. Sergi, M.-G. Martinoli, The Neuroinflammatory and Neurotoxic Potential of Palmitic Acid Is Mitigated by

Oleic Acid in Microglial Cells and Microglial-Neuronal Co-cultures, Mol. Neurobiol. 58 (2021) 3000–3014, https://doi.org/10.1007/s12035-021-02328-7.
[32] Z. Rafiee, A.M. García-Serrano, J.M.N. Duarte, Taurine Supplementation as a Neuroprotective Strategy upon Brain Dysfunction in Metabolic Syndrome and Diabetes, Nutrients 14 (2022), https://doi.org/10.3390/nu14061292.
[33] J.C. Russell, S.D. Proctor, S.E. Kelly, D.N. Brindley, Pair feeding-mediated changes in metabolism: stress response and pathophysiology in insulin-resistant, atherosclerosis-prone JCR:LA-cp rats, Am. J. Physiol. Endocrinol. Metab. 294 (2008) E1078–E1087, https://doi.org/10.1152/ajpendo.90257.2008.

ANEXO 2

Título: *Regional specific effect of saturated vs unsaturated fat on leptin receptor signalling in mice brain areas regulating feeding*.

Autores: Jesús Fernández Felipe, Lucía, L. López, Victoria Cano, Enrique Sánchez-Hita, Ana Belén Sanz Martos, Julie A.Chowen, Nuria del Olmo, Mariano Ruiz-Gayo y Beatriz Merino.

Publicado en: *Neuroscience letters* (ISSN: 0304-3940)

Factor de impacto: 3.197

Cuartil: Q3 (neurociencia)

Fecha de publicación: 2022

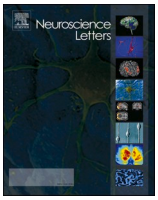
DOI: 10.1016/j.neulet.2022.136996

Neuroscience Letters 793 (2023) 136996

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet



Research article

Regional specific effect of saturated *vs* unsaturated fat on leptin receptor signalling in mice brain areas regulating feeding

Jesús Fernández-Felipe [a], Lucía L. López [a], Victoria Cano [a], Enrique Sánchez-Hita [a], A. Belén Sanz [b], Julie A. Chowen [c,d,e], Nuria Del Olmo [b], Mariano Ruiz-Gayo [a], Beatriz Merino [a,*]

[a] *AdipoBrain, Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, 28660 Madrid, Spain*
[b] *Departamento de Psicobiología, Facultad de Psicología, Universidad Nacional de Educación a Distancia (UNED), 28040 Madrid, Spain*
[c] *Departamento de Endocrinología, Hospital Infantil Universitario Niño Jesús, Instituto de Investigación La Princesa, 28009 Madrid, Spain*
[d] *Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III (CIBEROBN, ISCIII), 28029, Madrid, Spain*
[e] *IMDEA Alimentación, CEI UAM + CSIC, 28049 Madrid, Spain*

A B S T R A C T

Leptin receptors (LepR) are expressed in brain areas controlling food intake homeostasis, such as the hypothalamus, the hippocampus and the prefrontal cortex. In a previous study we reported that long-term intake of saturated and monounsaturated fat alters hypothalamic LepR signalling. The current study aims at investigating the effect of foods high in either saturated (SOLF) or monounsaturated fat (UOLF) on LepR functionality in the hippocampus and the prefrontal cortex. Male mice were placed on SOLF/UOLF (eight weeks), then treated with recombinant murine leptin (1 mg/kg). After 60 min, brain regions were dissected and processed for western blot of phosphorylated STAT3 (pSTAT3), Akt (pAkt) and AMPK (pAMPK). Levels of SOCS3 were also quantified. SOLF itself increased basal levels of pSTAT3, while UOLF impaired leptin-induced phosphorylation of both Akt and AMPK. SOCS3 levels were specifically increased by UOLF within the prefrontal cortex. Our results show that SOLF and UOLF differently affect LepR signalling within the hippocampus and the prefrontal cortex, which points to the complex effect of saturated and unsaturated fat on brain function, particularly in areas regulating food intake.

1. Introduction

Leptin receptors (LepR) activate transduction mechanisms that are shared with other cytokine receptors as well as with the insulin receptor. The best characterized signalling pathways coupled to LepR are the Janus kinase (JAK)-signal transducer and activator of transcription (STAT), the mitogen-activated protein kinase (MAPK), and the phosphatidylinositol-3-kinase (PI3K) – protein kinase B (Akt) pathways [1]. In addition, leptin is known to inactivate AMPK in the hypothalamus [2]. Brain leptin resistance triggered by regular intake of high-fat diets (HFD) is linked to the desensitization of leptin carriers, located both in the blood–brain barrier (BBB) and in the choroid plexus [3], and/or signalling pathways downstream of LepR [4], which has been related to the stimulation of the suppressor of cytokine signalling 3 (SOCS3) [4]. Leptin resistance linked to the intake of HFD has been classically associated to hyperleptinemia evoked by these diets [5,6], but a direct effect of fatty acids (FA) on leptin-sensitive neurons has also been reported [7].

In the brain, LepR are widely expressed in structures regulating feeding behaviour. In this context, as suggested by M.F. Dallman [8], the prefrontal cortex, the hippocampus and the hypothalamus are integral to a circuit connecting mindful, emotional, and homeostatic regulation of food intake; thus, leptin, together with other hormonal and metabolic inputs, would target these brain structures to regulate food intake and feeding behaviour by acting at different levels within this circuit.

Although the effect of HFD on the functionality of LepR in the hypothalamus has been extensively studied [9,10], data regarding LepR functionality in brain areas such as the prefrontal cortex or the hippocampus are lacking. In particular, the negative influence of palmitic acid on LepR signalling has attracted much attention [7,11], but studies aimed at characterizing the effect of other FA are scarce and offer controversial results [12]. The aim of the current study was to investigate the specific effect of monounsaturated (UOLF) *vs* saturated fat (SOLF) on LepR functionality in the above-mentioned areas.

* Corresponding author at: Universidad San Pablo–CEU, CEU Universities, Facultad de Farmacia, Campus de Montepríncipe, 28660, Boadilla del Monte, Madrid, Spain.
E-mail address: merpala.fcex@ceu.es (B. Merino).

https://doi.org/10.1016/j.neulet.2022.136996
Received 22 July 2022; Received in revised form 27 October 2022; Accepted 28 November 2022
Available online 5 December 2022

2. Methods and material

2.1. Animals and experimental protocol

Experiments were carried out in five-week-old male C57BL/6J mice (Charles River, France). Briefly, mice were housed under 12-h light/12-h dark cycle, in a temperature-controlled room (22 °C) with free access to water and chow, in accordance with the European Communities Council Directive (86/609/EEC) for the care and use of laboratory animals (PCD-CEU08-112-16). At the start of the experiment, the mice were divided into three groups with a similar mean body weight (BW), single housed, and given free access to the assigned diet for 8 weeks. These diets consisted of either standard chow (SD, 18 % energy from fat; Teklad global 2018, Harlan Laboratories, Spain, n = 18), a high-fat diet (HFD) enriched in monounsaturated fat (high oleic sunflower oil, **U**nsaturated **OiL**-enriched **F**ood, **UOLF**, 70 % energy from fat, n = 19), or a HFD enriched in saturated fat (palm kernel oil, **S**aturated **OiL**-enriched **F**ood, **SOLF**, 70 % energy from fat, n = 19). UOLF and SOLF were elaborated by mixing standard chow diet (60 %) and 40 % of either high-oleic sunflower oil or palm kernel oil, respectively, as previously described [13]. The nutritional composition is available in Plaza et al. (2019). Animals used in this study were the same used in a previous research [10].

2.2. Assessment of leptin resistance

Recombinant murine leptin (1 mg/kg) or saline were administered i.p at 0900 h to mice that had consumed either standard chow, UOLF or SOLF for 8 weeks. After 60 min, mice were killed by decapitation, and the hippocampus and prefrontal cortex prepared for western blotting. The dose of leptin was chosen in basis to previous studies of our group [14].

2.3. Western blotting

Western blotting was performed as previously reported [10]. Primary antibodies against pSTAT3, pAkt, pAMPK, and SOCS3 (1:250, Ref. 9131; 1:500, Ref. 9271; 1:500, Ref. 2531; and 1:500, Ref. 52113, respectively, Cell Signalling) were used. Clarity Western ECL Substrate (Bio-Rad Laboratories) was employed to visualize the chemiluminescent signal by ImageQuant Las 4000 Software (GE Healthcare Life Sciences, Barcelona, Spain). Phosphorylated proteins were normalized to their correspondent unphosphorylated forms (STAT3, 1:250, ref. Sc-483; Santa Cruz; Akt, 1:500, Ref. 9272 Cell Signalling; and AMPK, 1:500, Ref. 2532 Cell Signalling). SOCS3 was normalized to β-actin (1:5000, Ref. A-5316 Sigma Aldrich).

2.4. mRNA preparation and quantitative real-time PCR

Total RNA was extracted by using the Tri-Reagent protocol (Sigma, USA). cDNA was then synthesized from 1 μg total mRNA by using a high-capacity cDNA RT kit (Bio-Rad, CA). Quantitative RT-PCR was performed by using designed primer pairs (Integrated DNA Technologies, USA) for gene *Lepr* (forward 5′→3′ GCAGCAAAAGGAAGCATTGGA; reverse 5′→3′ GGTGAGGAGCAAGAGACTGG). SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, CA) was used for amplification according to the manufacturer's protocols, in CFX96 Real Time System (Bio-Rad, CA). Values were normalized to the housekeeping gene *Actb* (forward 5′→3′ TGGTGGGAATGGGTCAGAAGGACTC; reverse 5′→3′ CATGGCTGGGGTGTTGAAGGTCTCA) and *18 s* (forward 5′→3′ GGGAGCCTGAGAAACGGC; reverse 5′→3′ GGGTCGGGAGTGGGTAATTT). The Δ Δ C(T) method was used to determine relative expression levels. Statistics were performed using Δ Δ C(T) values [15]. All samples were run in duplicate.

2.5. Statistical analysis

All statistics were performed using GraphPad Prism software (GraphPad Software Inc. USA; Version 7.0a). Normal distribution and variance homogeneity were assessed by means of the Bartlett and Brown-Forsyte test. Leptin and diet effects were analysed by either two-way or one-way ANOVA with post-hoc Bonferroni correction. Data are expressed as mean ± S.E.M. and statistical significance was set at $p < 0.05$.

3. Results

3.1. Lepr gene expression is not modified by dietary treatment with high-fat diets

As appears illustrated in Fig. 1A and B, neither SOLF nor UOLF modified the expression of the *Lepr* gene in the hippocampus and prefrontal cortex.

3.2. SOLF increased basal STAT3 phosphorylation within the hippocampus and prefrontal cortex

Fig. 1C and D show the effect of dietary treatment and acute leptin on STAT3 phosphorylation within the hippocampus and the prefrontal cortex. In the hippocampus, two-ANOVA indicated an effect of leptin ($F_{(1,34)} = 53,39$; $P < 0.001$) and diet ($F_{(1,34)} = 15,80$; $P < 0.001$), as well as a significant interaction diet × leptin ($F_{(2,34)} = 3,28$; $P < 0.05$). In this brain area, leptin increased pSTAT3/STAT3 in control ($P < 0.001$) and UOLF mice ($P < 0.001$), but not in SOLF mice. Moreover, SOLF itself increased pSTAT3/STAT3 ($P < 0.001$).

In the prefrontal cortex**,** an effect of leptin was identified ($F_{(1,30)} = 29,72$; $P < 0.001$) that was dependent on dietary treatment ($F_{(2,30)} = 4,623$; $P < 0.05$).

3.3. UOLF abolished the effect of leptin on Akt phosphorylation within the hippocampus and prefrontal cortex

In the hippocampus, there was an effect of leptin ($F_{(1,34)} = 37,08$, $P < 0.001$) that was independent of diet within the hippocampus (Fig. 1E). In the prefrontal cortex (Fig. 1F), an effect of leptin was also detected ($F_{(1,30)} = 14,92$, $P < 0.001$). In this area, the effect of leptin depended on dietary treatment ($F_{(2,30)} = 3,255$, $P < 0.05$).

3.4. Influence of SOLF and UOLF on AMPK phosphorylation in the hippocampus and prefrontal cortex

In the hippocampus (Fig. 1G), a diet effect ($F_{(2,34)} = 3,255$, $P < 0.001$) as well as a leptin effect ($F_{(1,34)} = 87,42$, $P < 0.001$) were identified. The interaction was also significant ($F_{(2,34)} = 10,74$, $P < 0.001$). In the prefrontal cortex, only a leptin effect was identified that was independent of diet ($F_{(1,30)} = 16,66$, $P < 0.001$) (Fig. 1H).

3.5. UOLF increased SOCS3 in the prefrontal cortex

The effect of SOLF and UOLF on SOCS3 levels was analyzed in different brain areas with UOLF increasing SOCS3 only in the prefrontal cortex ($F_{(2,15)} = 20.39$, $p < 0.001$; Fig. 2)and with no effect of SOLF found.

4. Discussion

We have previously reported that SOLF- and UOLF-fed mice gain more weight than animals consuming chow, despite they all consume a similar number of calories. In addition, we have also observed that plasma leptin and insulin levels are higher in UOLF- than in SOLF-fed mice. Related to LepR signalling, we have reported that Akt, AMPK

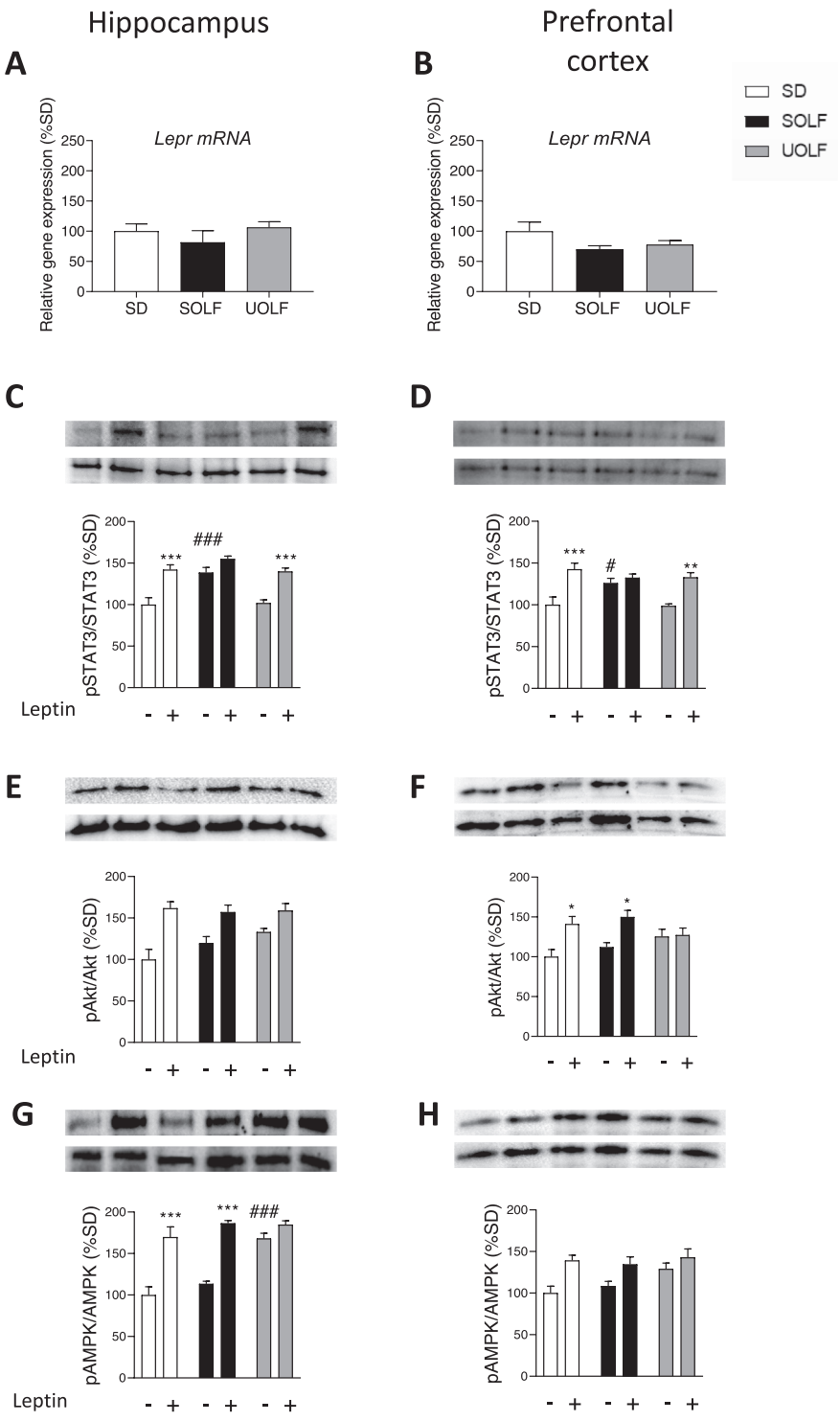


Fig. 1. Effect of SOLF and UOLF on *Lepr* expression and leptin receptor signalling in the hippocampus and prefrontal cortex. A and B: None of the diets influenced *Lepr* gene expression neither in the hippocampus nor in the prefrontal cortex. **Right panels** illustrate the effect of leptin on Tyr^{705}-STAT3 **(C),** Ser^{473}-Akt (**E**) and Thr^{172}-AMPK (**G**) phosphorylation in the HIP. **Left panels** show the effect of leptin on Tyr^{705}-STAT3 **(D),** Ser^{473}-Akt (**F**) and Thr^{172}-AMPK (**H**) phosphorylation within the prefrontal cortex. Representative blots are shown. Values represent the means ± S.E.M. of 6 animals *per* group. *P < 0.05, **P < 0.01, ***P < 0.001, compared to their matched saline groups. $^{\#}$P < 0.05, $^{\#\#\#}$P < 0.001, compared to SD-saline mice (Bonferroni's test).

and mTOR phosphorylation is impaired specifically by SOLF within the hypothalamus [10]. The current study shows that both SOLF and UOLF affect LepR signalling in the prefrontal cortex and hippocampus. This effect is not associated with any change on *Lepr* expression.

As already observed in the hypothalamus, UOLF did not alter the activation of STAT3 by leptin, despite that fact that UOLF increases plasma leptin levels more drastically than SOLF [13]. This finding, taken together with the increase of basal pSTAT3 induced by SOLF, shows that the adaptation of the hippocampus and prefrontal cortex to the diets is different to that found in the hypothalamus. These differences could be explained by inter-regional specificities in the accessibility of circulating leptin and/or in the sensitivity to particular FA. In fact, recent studies have shown that the access of leptin to circumventricular organs remains intact in obese mice [16], in contrast to other studies indicating that leptin leakage from blood toward the brain is impaired in obese mice [17]. This circumstance could be differently affected by SOLF and UOLF since diets enriched in saturated fat have been shown to enhance the permeability of the blood–brain barrier [18]. In regard to a specific responsiveness of brain areas to a particular FA, there are no data in the literature supporting our hypothesis. It is interesting to note that SOLF

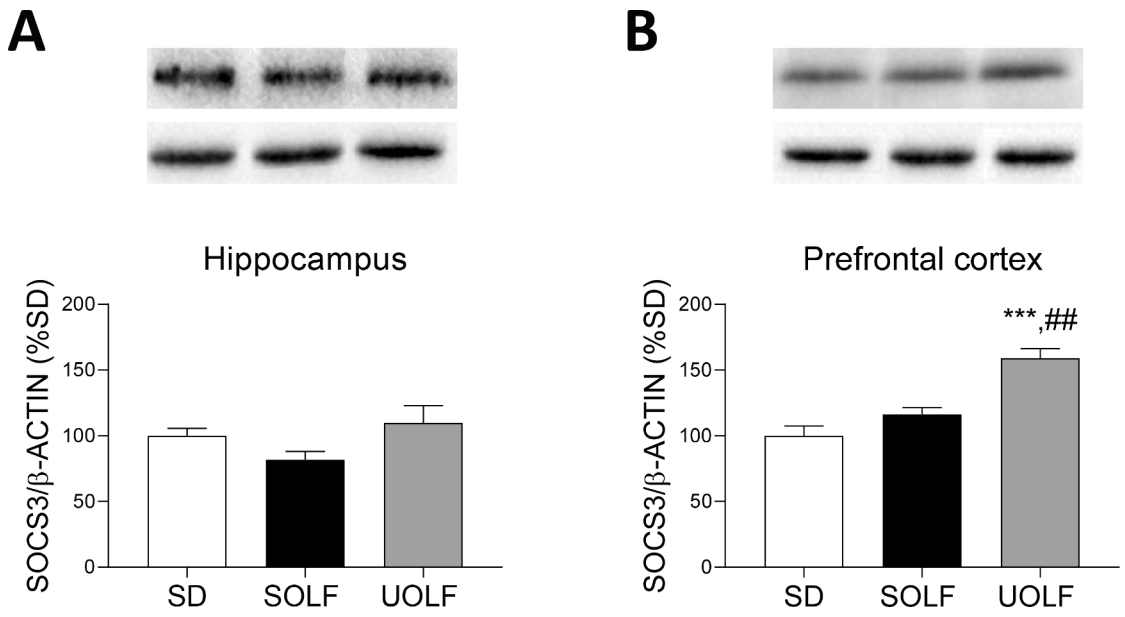


Fig. 2. Influence of dietary treatment on SOCS3 levels in hippocampus and prefrontal cortex. Values represent means ± S.E.M. of 6 animals *per* group. ***P < 0.001, compared to SD mice. ##P < 0.01, compared to SOLF mice (Bonferroni's test).

contains elevated amounts of lauric acid, which is almost absent in UOLF, and also that the palmitic acid/oleic acid ratio is different between SOLF and UOLF. Therefore, we speculate that either the presence/absence of lauric acid or a particular combination of FAs might account for the inter-regional differences detected in the current study. As a matter of fact, palmitic acid has been shown to promote inflammatory signalling [19,20], which could be related to the activation of STAT3 detected in SOLF animals [21]. It should be highlighted that we cannot conclude that SOLF uncouples LepR STAT3 signalling since SOLF itself increased pSTAT3 within the hippocampus and the prefrontal cortex, a circumstance that could mask the effect of leptin. Moreover, the lack of effect of SOLF on SOCS3 levels supports this possibility. Indeed, in a previous study we demonstrated that conventional HFD (containing both oleic and palmitic acids) do not alter the activation of STAT3 by leptin in the mice hippocampus [22].

A surprising finding of the current study is the deleterious effect of UOLF on Akt activation by leptin that contrasts with the result of a previous study showing that this pathway is specifically affected by SOLF in the hypothalamus. This discrepancy points to a region-dependent response to saturated and unsaturated FA and draws a complex picture concerning the effect of HFD on brain circuitry regulating feeding. Although more research is necessary, our data show that UOLF has an impact on the hippocampus and the prefrontal cortex that is coincident with the effect of conventional HFD [22]. Moreover, the effect of UOLF on Akt signalling could be related with the negative effect of this diet on synaptic plasticity within the hippocampus [23]. Nevertheless, palmitic acid [24] as well as saturated diets [10], have been shown to impair Akt signalling, which appears in contradiction with our current data showing that Akt signalling is hindered in mice consuming the unsaturated diet. Interestingly, SOCS3, which negatively regulates the Akt pathway [25,26] is increased in the prefrontal cortex of UOLF mice (no effect within the hippocampus). The overexpression of SOCS3 detected in this brain area of UOLF mice might be related to the elevated levels of both leptin and insulin, since these hormones have been shown to induce SOCS3 expression [27,28]; such an interaction seems to be absent in the hippocampus.

Leptin increased the phosphorylation levels of Thr^{172} on the catalytic alpha subunit of AMPK in control and SOLF mice but not in UOLF mice. This is striking as leptin has been shown to inactivate AMPK within the hypothalamus [2] and this observation also differs with our previous results showing that leptin inhibits AMPK both in control and UOLF mice in this brain area [10]. It should be noted that the effect of leptin on phosphorylated Thr^{172} AMPKα levels is complex, since it promotes AMPK phosphorylation in non-adipose tissues [29] but other studies have reported decreased AMPK activity in the hippocampus [30].

It is important to highlight that both SOLF and UOLF contain less proteins and carbohydrates than chow, which could influence food palatability and digestion as well as to have an impact on the endocrine system. Otherwise, increasing fat content implies a proportional deficit of proteins and/or carbohydrates. Such circumstances could contribute to the effect of SOLF/UOLF on LepR signalling. In any case, the fact that hypothalamic LepR signalling is specifically affected by SOLF [10] strongly supports the concept that fat content itself is not able to evoke changes in LepR signalling. Similar considerations would apply regarding an eventual deficit of carbohydrates and proteins.

Our current findings, revealing that both SOLF and UOLF alter LepR signalling within the hippocampus and the prefrontal cortex, are coherent with a previous study showing that these diets have a negative impact on mechanisms underlying memory; taken together with our previous study in the hypothalamus [10] our results would indicate that these diets trigger brain region specific effects.

CRediT authorship contribution statement

Jesús Fernández-Felipe: Writing – original draft. **Lucía L. López:** . **Victoria Cano:** . **Enrique Sánchez-Hita:** . **A. Belén Sanz:** . **Julie A. Chowen:** Conceptualization, Writing – original draft. **Nuria Del Olmo:** Conceptualization, Funding acquisition. **Mariano Ruiz-Gayo:** Conceptualization, Writing – original draft, Funding acquisition. **Beatriz Merino:** Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

[1] H. Münzberg, C.D. Morrison, Structure, production and signaling of leptin, Metabolism. 64 (2015) 13–23, https://doi.org/10.1016/j.metabol.2014.09.010.

[2] Y. Minokoshi, T. Alquier, N. Furukawa, Y.-B. Kim, A. Lee, B. Xue, J. Mu, F. Foufelle, P. Ferré, M.J. Birnbaum, B.J. Stuck, B.B. Kahn, AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus, Nature. 428 (2004) 569–574, https://doi.org/10.1038/nature02440.

[3] W.A. Banks, Leptin and the Blood-Brain Barrier: Curiosities and Controversies, Compr. Physiol. 11 (2021) 2351–2369, https://doi.org/10.1002/cphy.c200017.

[4] S.J. Park, Y. Yu, C.G. Zides, M.J. Beyak, Mechanisms of reduced leptin-mediated satiety signaling during obesity, Int. J. Obes. (Lond) 46 (2022) 1212–1221, https://doi.org/10.1038/s41366-022-01079-2.

[5] C. Bjørbaek, K. El-Haschimi, J.D. Frantz, J.S. Flier, The role of SOCS-3 in leptin signaling and leptin resistance, J. Biol. Chem. 274 (1999) 30059–30065, https://doi.org/10.1074/jbc.274.42.30059.

[6] Z.A. Knight, K.S. Hannan, M.L. Greenberg, J.M. Friedman, Hyperleptinemia is required for the development of leptin resistance, PLoS One. 5 (2010) e11376.

[7] A. Kleinridders, D. Schenten, A.C. Könner, B.F. Belgardt, J. Mauer, T. Okamura, F. T. Wunderlich, R. Medzhitov, J.C. Brüning, MyD88 signaling in the CNS is required for development of fatty acid-induced leptin resistance and diet-induced obesity, Cell Metab. 10 (2009) 249–259, https://doi.org/10.1016/j.cmet.2009.08.013.

[8] M.F. Dallman, Stress-induced obesity and the emotional nervous system, Trends Endocrinol. Metab. 21 (2010) 159–165, https://doi.org/10.1016/j.tem.2009.10.004.

[9] H. Münzberg, J.S. Flier, C. Bjørbæk, Region-Specific Leptin Resistance within the Hypothalamus of Diet-Induced Obese Mice, Endocrinology. 145 (2004) 4880–4889, https://doi.org/10.1210/en.2004-0726.

[10] J. Fernández-Felipe, M. Valencia-Avezuela, B. Merino, B. Somoza, V. Cano, A. B. Sanz-Martos, L.M. Frago, M.S. Fernández-Alfonso, M. Ruiz-Gayo, J.A. Chowen, Effects of saturated versus unsaturated fatty acids on metabolism, gliosis, and hypothalamic leptin sensitivity in male mice, Nutr. Neurosci. (2022) 1–14, https://doi.org/10.1080/1028415X.2022.2029294.

[11] S.C. Benoit, C.J. Kemp, C.F. Elias, W. Abplanalp, J.P. Herman, S. Migrenne, A.-L. Lefevre, C. Cruciani-Guglielmacci, C. Magnan, F. Yu, K. Niswender, B.G. Irani, W.L. Holland, D.J. Clegg, Palmitic acid mediates hypothalamic insulin resistance by altering PKC-theta subcellular localization in rodents, J. Clin. Invest. 119 (2009) 2577–2589, https://doi.org/10.1172/JCI36714.

[12] K. Mendoza-Herrera, A.A. Florio, M. Moore, A. Marrero, M. Tamez, S. N. Bhupathiraju, J. Mattei, The Leptin System and Diet: A Mini Review of the Current Evidence, Front. Endocrinol. (Lausanne) 12 (2021), 749050, https://doi.org/10.3389/fendo.2021.749050.

[13] A. Plaza, M. Antonazzi, J. Blanco-Urgoiti, N. Del Olmo, M. Ruiz-Gayo, Potential Role of Leptin in Cardiac Steatosis Induced by Highly Saturated Fat Intake during Adolescence, Mol. Nutr. Food Res. 63 (2019), https://doi.org/10.1002/mnfr.201900110.

[14] R. Guzmán-Ruiz, B. Somoza, M. Gil-Ortega, B. Merino, V. Cano, C. Attané, I. Castan-Laurell, P. Valet, M.S. Fernández-Alfonso, M. Ruiz-Gayo, Sensitivity of cardiac carnitine palmitoyltransferase to malonyl-CoA is regulated by leptin: Similarities with a model of endogenous hyperleptinemia, Endocrinology. 151 (2010) 1010–1018, https://doi.org/10.1210/en.2009-1170.

[15] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods. 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.

[16] L. Harrison, S.C. Schriever, A. Feuchtinger, E. Kyriakou, P. Baumann, K. Pfuhlmann, A.C. Messias, A. Walch, M.H. Tschöp, P.T. Pfluger, Fluorescent blood-brain barrier tracing shows intact leptin transport in obese mice, Int. J. Obes. (Lond) 43 (2019) 1305–1318, https://doi.org/10.1038/s41366-018-0221-z.

[17] B. Burguera, M.E. Couce, G.L. Curran, M.D. Jensen, R.V. Lloyd, M.P. Cleary, J. F. Poduslo, Obesity is associated with a decreased leptin transport across the blood-brain barrier in rats, Diabetes 49 (2000) 1219–1223, https://doi.org/10.2337/diabetes.49.7.1219.

[18] C. Li, L. Shi, Y. Wang, C. Peng, L. Wu, Y. Zhang, Z. Du, High-fat diet exacerbates lead-induced blood-brain barrier disruption by disrupting tight junction integrity, Environ. Toxicol. 36 (2021) 1412–1421, https://doi.org/10.1002/tox.23137.

[19] S. Gupta, A.G. Knight, S. Gupta, J.N. Keller, A.J. Bruce-Keller, Saturated long-chain fatty acids activate inflammatory signaling in astrocytes, J. Neurochem. 120 (2012) 1060–1071, https://doi.org/10.1111/j.1471-4159.2012.07660.x.

[20] N. Yanguas-Casás, A. Crespo-Castrillo, M.L. de Ceballos, J.A. Chowen, I. Azcoitia, M.A. Arevalo, L.M. Garcia-Segura, Sex differences in the phagocytic and migratory activity of microglia and their impairment by palmitic acid, Glia. 66 (2018) 522–537, https://doi.org/10.1002/glia.23263.

[21] G.E. Tyzack, C.E. Hall, C.R. Sibley, T. Cymes, S. Forostyak, G. Carlino, I.F. Meyer, G. Schiavo, S.-C. Zhang, G.M. Gibbons, J. Newcombe, R. Patani, A. Lakatos, A neuroprotective astrocyte state is induced by neuronal signal EphB1 but fails in ALS models, Nat. Commun. 8 (2017) 1164, https://doi.org/10.1038/s41467-017-01283-z.

[22] I. Valladolid-Acebes, A. Fole, M. Martín, L. Morales, M.V. Cano, M. Ruiz-Gayo, N. Del Olmo, Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin? Neurobiol. Learn. Mem. 106 (2013) 18–25, https://doi.org/10.1016/j.nlm.2013.06.012.

[23] J. Fernández-Felipe, B. Merino, A.B. Sanz-Martos, A. Plaza, A. Contreras, V. Naranjo, L. Morales, J.A. Chowen, V. Cano, M. Ruiz-Gayo, N. Del Olmo, Saturated and unsaturated fat diets impair hippocampal glutamatergic transmission in adolescent mice, Psychoneuroendocrinology. (2021) 105429. 10.1016/j.psyneuen.2021.105429.

[24] L. Cheng, Y. Yu, A. Szabo, Y. Wu, H. Wang, D. Camer, X.-F. Huang, Palmitic acid induces central leptin resistance and impairs hepatic glucose and lipid metabolism in male mice, J. Nutr. Biochem. 26 (2015) 541–548, https://doi.org/10.1016/j.jnutbio.2014.12.011.

[25] K. Ueki, T. Kondo, Y.-H. Tseng, C.R. Kahn, Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 10422–10427, https://doi.org/10.1073/pnas.0402511101.

[26] L. Cao, Z. Wang, W. Wan, Suppressor of Cytokine Signaling 3: Emerging Role Linking Central Insulin Resistance and Alzheimer's Disease, Front. Neurosci. 12 (2018) 417, https://doi.org/10.3389/fnins.2018.00417.

[27] J.K. Howard, J.S. Flier, Attenuation of leptin and insulin signaling by SOCS proteins, Trends Endocrinol. Metab. 17 (2006) 365–371, https://doi.org/10.1016/j.tem.2006.09.007.

[28] M.B. Ernst, C.M. Wunderlich, S. Hess, M. Paehler, A. Mesaros, S.B. Koralov, A. Kleinridders, A. Husch, H. Münzberg, B. Hampel, J. Alber, P. Kloppenburg, J. C. Brüning, F.T. Wunderlich, Enhanced Stat3 activation in POMC neurons provokes negative feedback inhibition of leptin and insulin signaling in obesity, J. Neurosci. Off. J. Soc. Neurosci. 29 (2009) 11582–11593, https://doi.org/10.1523/JNEUROSCI.5712-08.2009.

[29] Y. Minokoshi, Y.-B. Kim, O.D. Peroni, L.G.D. Fryer, C. Müller, D. Carling, B. B. Kahn, Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase, Nature 415 (2002) 339–343, https://doi.org/10.1038/415339a.

[30] Y. Dagon, Y. Avraham, I. Magen, A. Gertler, T. Ben-Hur, E.M. Berry, Nutritional status, cognition, and survival: a new role for leptin and AMP kinase, J. Biol. Chem. 280 (2005) 42142–42148, https://doi.org/10.1074/jbc.M507607200.